Cerebral Microbleeds as a Marker of Small Vessel Disease: New Insights from Neuro-imaging and Clinical Studies in Stroke Patients

by

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I, Simone M. Gregoire, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Simone M Gregoire

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ABSTRACT

Introduction: A portfolio of studies is presented aimed at understanding the clinical and pathophysiological significance of cerebral microbleeds (CMBs) in stroke patients. CMBs are the radiological marker of microscopic haemosiderin deposits on iron-sensitive MRI sequences (mainly gradient-recalled echo [GRE] T2* MRI). They are common in patients with cerebrovascular disease and are hypothesised to be a biomarker for brain small vessel diseases, including hypertensive arteriopathy and cerebral amyloid angiopathy (CAA). Important questions relating to CMBs include their use as a prognostic marker for antithrombotic-related intracerebral haemorrhage (ICH) and cognitive impairment. Our aims were to address the pathophysiological and clinical relevance of CMBs using longitudinal, case-control and cross-sectional studies.

Methods: Patients were ascertained from prospective databases of admissions to the stroke service at the National Hospital for Neurology and Neurosurgery and at University College London Hospital's (UCLH) NHS Trust. Magnetic resonance imaging data was collected and analysed for markers of small vessel disease including CMBs. Clinical and radiological associations of CMBs were determined using appropriate statistical tests.

Objectives: First, ways of improving microbleed detection and reporting were explored through the development of a visual rating scale (the Microbleed Anatomical Rating Scale, MARS) aimed at reliably rating CMBs. Second, the prognostic relevance of CMBs was investigated for antiplatelet-related ICH in a case-comparison study. Third, the detection of new CMBs over time and the factors that influence this were explored. Fourth, the impact of CMBs on cognitive impairment was studied in a cross-sectional study. Finally, the association between CMBs and acute silent ischaemia on diffusion-weighted MRI was investigated via a multi-centre cross-sectional MRI study of patients with ICH.

Main findings: 1. MARS is a reliable scale with good intra- and inter-rater agreement for rating CMBs presence and number in any brain location. 2. Lobar CMBs, especially if numerous, are a risk factor for antiplatelet-related ICH independent of the extent of white matter changes. 3. CMBs accumulate over time in stroke patients, and the risk is related to baseline systolic blood pressure. 4. Lobar CMBs are an independent predictor of frontal executive impairment; this suggests that CAA is a potential underlying contributor to cognitive impairment. 5. Silent acute infarcts are frequent in patients within 3 months of ICH, especially in those with probable CAA, and are associated with markers of small vessel disease severity, including CMBs.

Conclusion: These studies provide new information on detection, clinical impact and associations of CMBs in stroke patients. They suggest that CMBs have useful roles in understanding pathophysiology, diagnosis and prognosis in patients with small vessel diseases. Further studies are required to determine the direct therapeutic consequences of CMBs, but the present work suggests several promising areas for future research.

LIST OF PUBLICATIONS

First author:

1. **Gregoire SM**, Scheffler G, Jäger HR, Yousry TA, Brown MM, Kallis C, Cipolotti L, Werring DJ. Strictly lobar microbleeds are associated with executive impairment in patients with ischaemic stroke or transient ischemic attack. *Stroke* 2013; 44: 1267-1272.

2. **Gregoire SM**, Smith K, Jäger HR, Benjamin M, Kallis C, Brown MM, Cipolotti L, Werring DJ. Cerebral microbleeds and long-term cognitive outcome: longitudinal cohort study of stroke clinic patients. *Cerebrovasc Dis* 2012; 33: 430-435.

3. **Gregoire SM**, Charidimou A, Gadapa N, Dolan E, Antoun N, Peeters A, Vandermeeren Y, Laloux P, Baron J-C, Jäger HR, Werring DJ. Acute ischaemic brain lesions in intracerebral haemorrhage: multicentre cross-sectional MRI study. *Brain* 2011; 134: 2376-2386.

4. **Gregoire SM**, Jäger HR, Yousry TA, Kallis C, Brown MM, Werring DJ. Brain microbleeds as a potential risk factor for antiplatelet-related intracerebral haemorrhage: hospital-based, case-control study. *J Neurol, Neurosurg Psychiatry* 2010; 81: 679-684.

5. **Gregoire SM**, Werring DJ, Chaudhary UJ, Thornton JS, Brown MM, Yousry TA, Jäger HR. Choice of echo time on GRE T2*-Weighted MRI influences the classification of brain microbleeds. *Clin Radiol* 2010; 65: 391-394.

6. **Gregoire SM**, Brown MM, Kallis C, Jäger HR, Yousry TA, Werring DJ. MRI detection of new microbleeds in ischaemic stroke patients: 5-year cohort follow-up study. *Stroke* 2010; 41: 184-186.

7. **Gregoire SM**, Chaudhary UJ, Brown MM, Yousry TA, Kallis C, Jäger HR, Werring DJ. The Microbleed Anatomical Rating Scale (MARS): reliability of a tool to map brain microbleeds. *Neurology* 2009; 73: 1759-1766.

Second author:

1. Charidimou A, **Gregoire SM**, Werring DJ. Letter by Charidimou et al. regarding article, "Blood pressure reduction, decreased diffusion on MRI, and outcomes after intracerebral hemorrhage". *Stroke* 2012; 43: e34; author reply e35.

2. Werring DJ, **Gregoire SM**, Cipolotti L. Cerebral microbleeds and vascular cognitive impairment. *J Neurol Sci* 2010; 299: 131-135.

Other papers:

1. Charidimou A, Peeters A, Fox Z, **Gregoire SM**, Vandermeeren Y, Laloux P, Jäger HR, Baron JC, Werring DJ. Spectrum of transient focal neurological episodes in cerebral amyloid angiopathy: multicentre magnetic resonance imaging cohort study and meta-analysis. *Stroke* 2012; 43: 2324-2330.

2. Dassan P, Brown MM, **Gregoire SM**, Keir G, Werring DJ.Association of Cerebral Microbleeds in Acute Ischemic Stroke With High Serum Levels of Vascular Endothelial Growth FactorMicrobleeds and VEGF in Acute Ischemic Stroke. *Arch Neurol* 2012; 28: 1-4.

3. James TH, Ramadan H, **Gregoire SM**, Mufti S,Lipman G, Jäger HR, Losseff N, Werring DJ.Can cerebral microbleeds cause an acute stroke syndrome? *Neurol Clin Pract* 2011; 1: 75-77.

4. Seghier ML, Kolanko MA, Leff AP, Jäger HR, **Gregoire SM**, Werring DJ. Microbleed Detection Using Automated Segmentation (MIDAS): A New Method Applicable to Standard Clinical MR Images. *PLoS One* 2011; 6: e17547.

5. Lovelock CE, Cordonnier C, Naka H, Al-Shahi Salman R, Sudlow CL, Jackson C, Sorimachi T, Werring DJ, **Gregoire SM**, Imaizumi T, Lee SH, Briley D, Rothwell PM. Antithrombotic drug use, cerebral microbleeds and intracerebral haemorrhage: a systematic review of published and unpublished studies. *Stroke* 2010; 41: 1222-1228.

Book Chapters:

1. **Gregoire SM** & Werring DJ 2011. Defining and mapping cerebral microbleeds. *In: Cerebral microbleeds: Pathophysiological to clinical practice.* Edited by David J Werring, New York: Cambridge University Press.

2. **Gregoire SM** & Werring DJ 2011. Other clinical manifestations of cerebral microbleeds. *In: Cerebral microbleeds: Pathophysiological to clinical practice.* Edited by David J Werring, New York: Cambridge University Press.

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ABBREVIATIONS

AD	Alzheimer's Disease
AF	Atrial Fibrillation
APOE	Apolipoprotein E
APP	Amyloid Precursor Protein
Aβ	Amyloid β
BAFTA	Birmingham Atrial Fibrillation Treatment of the Aged Study
BOMBS	Brain Observer Microbleed Scale
BP	Blood Pressure
CAA	Cerebral Amyloid Angiopathy
CADASIL	Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy
CARASIL	Cerebral Autosomal Recessive Arteriopathy with Subcortical Infarcts and Leukoencephalopathy
CI	Confidence Interval
CMB	Cerebral Microbleed
CSF	Cerebrospinal Fluid
CT	Computer Tomography
DPWM	Deep and Periventricular White Matter
DTI	Diffusion-Tension Imaging
DWI	Diffusion Weighted Imaging
EPI	Echo Planar Imaging
FA	Flip Angle
FLAIR	Fluid-Attenuated Inversion Recovery
fMRI	Functional MRI
FSE MRI	Fast Spin Echo MRI
GRE T2*-Weighted	Gradient Recalled Echo T2*-Weighted
HERNS	Hereditary Endotheliopathy with Retinopathy Nephropathy and Stroke
ICH	Intracerebral Haemorrhage
IHD	Ischaemic Heart Disease
INR	International normalisation ratio
IQ	Interquartile Range
IS	Ischaemic Stroke
MARS	Microbleed Anatomical Rating Scale
MCI	Mild Cognitive Impairment
MMSE	Mini Mental State Examination
MRI	Magnetic Resonance Imaging
NHNN	National Hospital for Neurology and Neurosurgery

NIHSS	National Institute of Health Stroke Scale
OR	Odds Ratio
PACS	Picture Archiving and Communications System
PET	Positron Emission Tomography
RF	Radiofrequency
RR	Relative Risk
SAH	Subarachnoid Haemorrhage
SD	Standard Deviation
SE	Spin Echo
SPIRIT	Stroke Prevention in Reversible Ischaemia Trial
SPSS	Statistical Package for the Social Sciences
SWI	Susceptibility-Weighted Imaging
TE	Time to Echo
TIA	Transient Ischaemic Attack
TOAST	Trial of Org 10172 in Acute Stroke
TR	Time to Repeat
UCLH	University College London Hospital
VCI	Vascular Cognitive Impairment
WML	White Matter Lesions

PREFACE

Research in recent years, including this work, suggests that cerebral microbleeds (CMBs) may have a greater impact on ageing societies than previously thought. Rather than being focused on one particular aspect or disease related to CMBs, this work covers multiple aspects of CMBs in the context of cerebral small vessel diseases. I have therefore chosen to summarise current knowledge on the considerable number of relevant topics in a comprehensive and detailed introduction. The latter encompasses all relevant topics covered in this thesis including the definitions and characteristics of the major small vessel diseases, the radiological methods to detect CMBs, and their clinical implications. In such a broad spectrum of overlapping topics, repetition is inevitable, but I have tried to keep it to a minimum. The thorough and wide-ranging historical introduction aims to provide the essential background to allow an understanding of current concepts of small vessel diseases and CMBs.

The thesis is in 2 sections; the first is an introduction on small vessel diseases and CMBs, and the second is a systematic report of the results of our main studies. I then discuss and summarise our findings in the conclusion and suggest ideas for future research on CMBs. As a final note, the terminologies used in the thesis are based on the most recent standards for reporting small vessel diseases and are the most commonly used term to report microbleeds, 'cerebral microbleeds' or CMBs. Like many authors, I used the term 'cerebral small vessel disease' to indiscriminately describe both the neuroimaging correlates and the underlying small vessel pathology, but created separate sections to describe these aspects for ease of reading and understanding.

PART 1: INTRODUCTION TO SMALL VESSEL DISEASE AND CEREBRAL MICROBLEEDS

Chapter 1 - Small vessel disease: definition and clinical relevance

The objectives of this chapter are to describe the different types of brain small vessel diseases, to understand their neuropathology, neuroimaging and clinical features.

1.1 Small vessel disease: definitions and classification

The term cerebral small vessel disease refers to a group of pathological processes affecting the small arteries, arterioles, venules and capillaries of the brain. Two main processes have been identified: (1) age-related and hypertension-related small vessel arteriopathy affecting the deep perforating small vessels, and (2) cerebral amyloid angiopathy (CAA), affecting mainly superficial cortical and leptomeningeal small vessels. The consequences of these processes on the brain parenchyma are mainly lesions located in the subcortical structures and include lacunar infarcts, white matter lesions (WML, leukoaraiosis), intracerebral haemorrhages (ICHs) and cerebral microbleeds (CMBs). Unlike large vessels, small vessels are not easily visualised *in vivo*. Therefore, these parenchymal lesions are used as surrogate markers of the underlying process, and the term small vessel disease has been adopted to describe these lesions. They are easily detected by neuroimaging and their characteristic imaging appearances have been extensively studied (Wardlaw et al., 2013). Clinically, small vessel diseases have a range of presentations and are a leading cause of cognitive decline and functional impairment in the elderly. They are one of the main targets for preventive and treatment strategies and should be taken into account in clinical practice and therapeutic trials.

Anatomically, cerebral arterial small vessels have two origins: superficially, they stem from the subarachnoid circulation as the terminal vessels of medium-sized cortical and leptomeningeal arteries, which originate from larger arteries; and at the base of the brain, they stem directly from the large vessels as small arterial perforators or lenticulostriate arteries (Pantoni, 2010). These two systems converge towards each other and after having passed through the cortical layers and the deep grey structures, respectively, merge in the deepest areas of the subcortical white matter where there is a ‘borderzone’ or watershed area (Figure 1).

A B

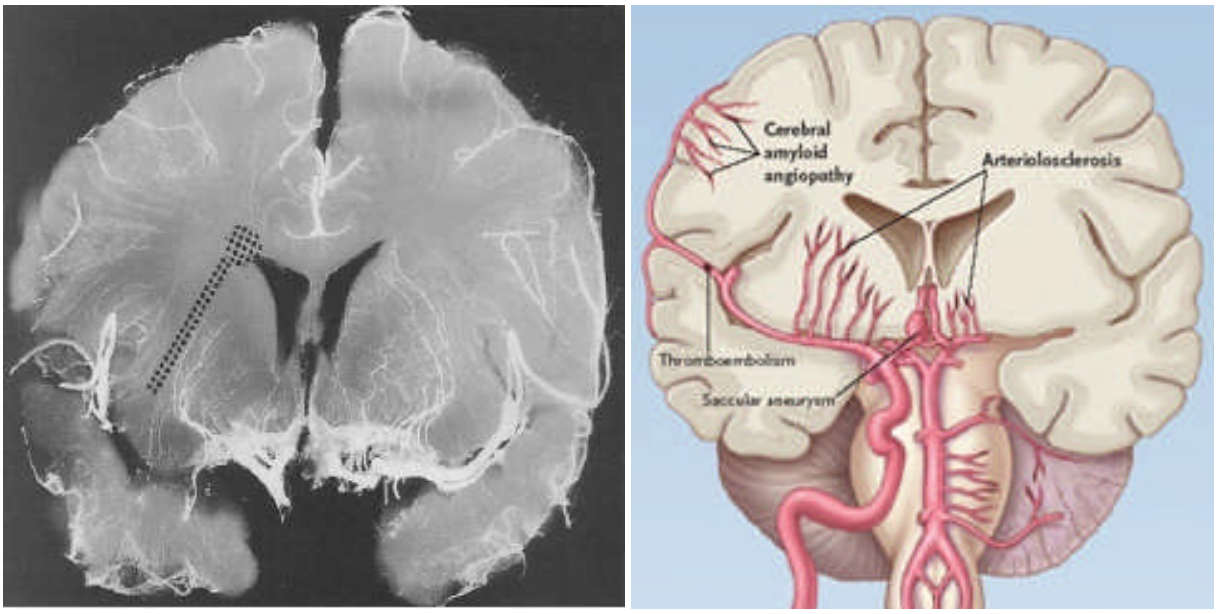


Figure 1.The brain vasculature and the main disease processes.

A. Coronal post-mortem microangiogram showing the distribution of the lenticulostriate arteries and terminal superficial arteries. Watershed infarcts are located in the paraventricular zone (circular dotted area) and the border between the lenticulostriate arteries and the short insular penetrating branches (line of dots). From (Veglio et al., 2009).
B. Schematic representation of the brain vasculature showing the main disease processes that can affect the small vessels: CAA affects capillaries, small to medium-sized arteries of the leptomeninges and cerebral cortex, while arteriolosclerosis affects the deep perforating arteries. From Greenberg, 2006

Small vessel diseases are systemic disorders that can affect various organs and areas of the body. One approach to classify the different types of small vessel diseases is shown in Table 1. This thesis will mainly focus on those small vessel diseases where the brain is one of the main targets and which are the most prevalent forms; arteriolosclerosis and CAA, with a specific interest in the sporadic form of CAA. Both of these processes have been linked with the presence of CMBs in the brain which are the main focus of this work.

Table 1. Aetiopathogenic classification of cerebral small vessel disease

(adapted from Pantoni, 2010)

Type	Characteristics	Name(s)
Age-related and hypertension related arteriopathy (also termed arteriolosclerosis or 'hypertensive arteriopathy')	Age-related and vascular-risk-factor-related, affects the deep perforators. Loss of smooth muscle cells, wall thickening	Fibrinoid necrosis Lipohyalinosis Microatheroma Microaneurysms Segmental arterial disorganisation
CAA	Amyloid-β deposition in small arteries, capillaries and veins (cortical and leptomeningeal)	Sporadic CAA Hereditary CAA (Dutch type) Familial non-Aβ CAA: British, Danish, Icelandic
Inherited or genetic	Inherited small vessel diseases distinct from CAA	e.g. CADASIL, CARASIL MELAS Fabry disease Small vessel diseases caused by COL4A1 mutations Hereditary multi-infarct dementia of the Swedish type Hereditary cereboretinal vasculopathy Hereditary endotheliopathy with retinopathy Nephropathy and stroke
Inflammatory and immunologically mediated	Heterogeneous, rare, usually systemic, presence of inflammatory cells in vessel walls (vasculitis)	e.g. Wegener's granulamatosis Churg-Strauss syndrome Microscopic polyangiitis Henoch-Schonlein purpura Cryoglobulinaemic vasculitis Primary angiitis of the CNS
Venous collagenosis	Pathological appearance of veins and venules located next to the lateral ventricles	
Other small vessel diseases	-	e.g. post-radiation angiopathy non-amyloid microvessel degeneration in Alzheimer's disease

1.2 Historical background

1.2.1 History of lacunes and white matter lesions

Since the beginning of the 19th century, physicians and scientists have had great interest in trying to understand the causes of ischaemic and haemorrhagic stroke. Post-mortem examination of the brain, a common technique since the 17th century, was used by pathologists to report neuropathological findings from patients who had died from what was then called *cerebral apoplexia*. Amédée Dechambre, a French physician at the Salpétrière Hospital in Paris, made breakthrough finding in 1838 which was a turning point in the history of small vessel diseases. He first reported the presence of 'lacunes' in stroke survivors, referring to the empty space or cavity (*lacuna* in Latin means tiny hole or cavity) remaining after liquefaction and re-absorption of the core of a small stroke. This generated a research boom in the field of small vessel diseases. In the following decades many people reported their own observations of lacunes and other small vessels-related lesions which set the foundation for our current understanding of small vessel disease pathology (Figure 2). Five years later, Maxime Durand-Fardel (1816-1899) confirmed Dechambre's observations and provided a more detailed description of lacunes as healed, small infarcts. He also described the *atrophie interstitielle du cerveau* in elderly patients and coined the term 'état criblé' (meaning 'tissue riddled with holes') to describe the dilatations of the perivascular spaces associated with small vessel disease. In 1894, Alzheimer and Binswanger both described the 'arteriosclerotic brain atrophy', a form of vascular dementia (VaD) characterised by 'miliary apoplexies' (most likely multiple lacunar strokes) affecting the basal ganglia, internal capsule and white matter of the centrum semi-ovale.

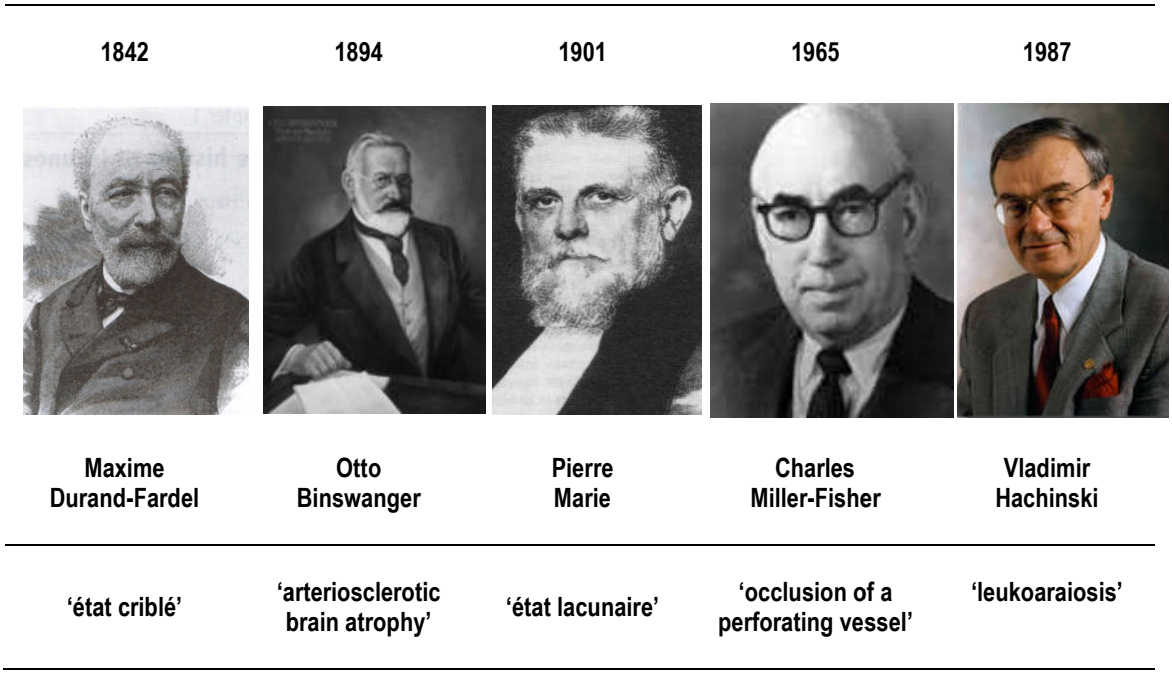


Figure 2. Scientists who provided a significant contribution to the understanding of small vessel disease, with associated descriptive terms

Pierre Marie (1853-1940) provided the best clinicopathological correlation study of lacunes whilst working at the Hospice for the Elderly in Bicêtre, near Paris. In 1901 he published his famous paper *Des foyers lacunaires de désintégration et les différents autres états cavitaires du cerveau*, in which he coined the term 'état lacunaire'. This term was used to describe a clinical picture characterised by recurrent episodes of motor deficit in the elderly, often with partial resolution, and accompanied by pseudobulbar palsy, incontinence and small-steps gait. These are still the hallmarks of the 'small vessel disease syndrome' described in modern clinical studies (Basile et al., 2006). The brains of these patients showed multiple lacunes as small softenings caused by atherosclerosis. It is highly likely that the 'arteriosclerotic brain atrophy' observed by Alzheimer and Binswanger was equivalent to Pierre Marie's 'état lacunaire' (Román, 2002). Nevertheless, the use of the two terms 'état lacunaire' and 'état criblé' created much confusion in subsequent works. In the 1960s, Fisher provided illuminating clinicopathological studies describing lacunes as we know them today: small deep cerebral infarcts secondary to the hypertensive occlusive disease of small vessels.

Leukoaraiosis, a word derived from the Greek 'leuko' meaning white and referring to the white matter, and 'araiosis' meaning rarefaction, was first coined by Vladimir Hachinski in 1987 to describe the radiological images of loss of density of the periventricular white

matter associated with hypertension as observed by computerised tomography (CT) (Hachinski et al., 1987). The introduction of this terminology was aimed at preventing confusion with a specific pathological process that had no criteria or definition consensus (so called Binswanger's disease) and to avoid attributing an explicit clinical cause or significance to these WML. It is possible that the *atrophie interstitielle du cerveau* described by Dechambre was the first description of what became known as leukoaraiosis.

Twenty-five years later, the amount of data on the clinical and pathological correlates of small vessel disease has enormously increased. The introduction of new imaging techniques of the brain — initially CT scanning then, since the 1980s, magnetic resonance imaging (MRI) — has enabled the increasingly sensitive detection of WML *in vivo*. The use of improved diagnostic techniques, e.g. diffusion-tension imaging (DTI) and functional MRI (fMRI), has contributed to a deeper understanding of the pathophysiology of small vessel diseases and their clinical correlates (Pantoni, 2010). These techniques have also demonstrated that etiologies other than hypertension can cause lacunes and leukoaraiosis. There are now a few high field studies showing that small vessels can be seen *in vivo* (Harb et al., 2013; Schreiber et al., 2013). The improved definitions and characteristics of small vessel-related lesions arising from these new developments will be detailed in the next sections.

1.2.2 History of cerebral amyloid angiopathy

Amyloid is an *in vivo* material, with a protein fibrillar structure (seen on electron microscopy), and typical x-ray diffraction pattern, an affinity for some histological stains, especially Congo red. Disease associated with, or caused by, amyloid, is termed 'amyloidosis'. Systemic amyloidosis is derived from a plasma protein, while localized amyloid derives from proteins at the site of the disease. According to Belokrenitzky, the first observations of *dégénérescence amyloide* date back from the 17th century and consist of pathological studies of fragments of spleen, kidney and liver with a tissue aspect described as waxy (Belokrenitzky, 1911). In 1853, Virchow created the term *dégénérescence amyloide* because the substance coloured with iodine had a brown mahogany colour that resembles the colour of starch (*amidon*, in French) when treated with the same metalloid. Wild (Goldberg, 1950; Reddy, 1951) and Wilks (Goldberg, 1950; Higgins and Higgins, 1950) provided the first descriptions of cases of 'primitive amyloidosis'. The proteinic nature of the substance is due to Kekule and Friedrich in 1859

(Martin, 1947). The association with multiple myeloma was first reported by Askanazy in 1904 (Goltz, 1952).

In the early 20th century, several colorations and techniques were used to detect amyloid in patients with 'primitive amyloidosis', based on the strong affinity of amyloid deposits for silver and their property to enhance with red Congo and methyl or cresyl violet (Schwarz, 1970). These techniques had only been scarcely used to detect amyloid in the cerebral tissue. The first reports of cerebral amyloid were made in patients affected by various types of intracerebral tumours (Fischer and Holfelder, 1930; Morgenstern, 1935). Amyloid was further detected in patients with Alzheimer's disease (AD) using Bielschowsky's silver technique, based on the strong affinity of the substance for silver (Allsop, 2000). The technique revealed dense bundles of fibrils within the nerve cells (neurofibrillary tangles) and numerous focal lesions within the cerebral cortex that were subsequently called 'senile plaques'. Bielschowsky stated that the substance found in senile plaques represented amyloid on the basis of its iodine reaction and metachromasia with methyl violet. In 1942, Divry published a neuropathological series of 35 brains with dementia and 12 brains with Alzheimers' disease and demonstrated the presence of vascular amyloid ('dyshoric angiopathy') within small cortical and/or meningeal vessels in 20 out of 35 demented patients, with a predilection for the occipital lobes (Divry, 1941). The amyloid present within vessels had the characteristics of the amyloid substance observed within senile plaques (e.g. congophilia, birefringence under polarised light and a crystalloid nature). This confirmed for the first time that the *drusige Entartung* of Scholz (dyshoric angiopathy) (Scholz, 1938) and senile plaques were two processes involving amyloid (Divry, 1941). A decade later, Pantelakis provided the first large clinico-pathological study of 82 patients with various psychiatric conditions of which a third had vascular amyloid which he called 'congophilic angiopathy' (Pantelakis, 1954). In contrast with 'primitive amyloidosis', vascular amyloid was absent in other organs. The amyloid substance was present within the cortical and/or meningeal vessels and in senile plaques as in Divry's cases. Pantelakis noted that all his studies related to patients who had been elderly and demented, and that the process was independent of the presence and severity of large vessel atherosclerosis.

Subsequent electron microscopic studies revealed that the amyloid in both senile plaques and vessel walls was composed of extracellular fibrils identical to amyloid fibrils in other organs (Schlote, 1965; Terry et al., 1964). However, amyloid was not detected in the blood vessels or the interstitium of any other organs, suggesting a localised form of amyloidosis

and confirming Pantelakis' findings (Ishii et al., 1984). Except in rare circumstances (Benson et al., 1977), amyloid deposition in this condition only affects the brain.

In light of these findings, there are now three recognised forms of amyloid deposition in the central nervous system. These are the amyloid core of senile plaques, deposits in the arterial and arteriolar walls ('congophilic angiopathy' of Pantelakis), and deposits in the capillary and arteriolar walls with extension into the surrounding brain parenchyma (*drusige Entartung* of Scholz, or dyshoric angiopathy) (Ishii et al., 1984). The latter two conditions are not mutually exclusive and are together called 'amyloid angiopathy'. It is frequently observed in the cerebral vessels of elderly patients and is almost invariable in AD. More recent immunocytochemical studies have shown that AD amyloid and vascular amyloid were composed of two different forms of amyloid. The amyloid plaques of AD are predominantly composed of a 42 amino acid residue fragment (Aβ42) whereas vascular amyloid is mostly composed of a more soluble 40 amino acid fragment (Aβ40), suggesting different pathophysiological mechanisms for its deposition (Charidimou et al., 2012b).

1.3 Pathological features

1.3.1 Age-related and hypertension-related small vessel arteriopathy

Hypertensive small vessel disease affects arteries, arterioles of various sizes and capillaries. Vascular changes induced by hypertension are associated with two recognised forms of small vessel vasculopathy: (1) arteriosclerosis and (2) atherosclerosis. Both processes are often combined. The former describes a generalised hardening of the arterial vessel wall (also termed fibrohyalinosis); the latter is a form of arteriosclerosis with deposition of lipids (atheroma) in the endothelium (Brandner, 2011). Theoretically, hypertension causes a forced dilation of resistance vessels, resulting in a loss of autoregulation of the arteries. This exposes smaller arteries and capillaries to excessive, unregulated blood pressure (BP) and results in a disruption of the blood-brain barrier and vasogenic oedema (Ferrer et al., 2008). Chronic hypertension and BP dysregulation causes structural alteration of the vessel wall with deposition of plasma proteins including fibrin, and damage to the smooth muscle cells in the media of small arteries. Chronic endothelial damage and platelet activation trigger the coagulation cascade, the formation of microthrombi and small vessel ischaemia (Brandner, 2011).

Typically, the vessels affected by small vessel disease are between 40 and 900 mm in size. The arteries affected are superficial perforating vessels and basal (deep) perforating vessels (Figure 1). Four types of structural change have been described in hypertension-related small vessel disease, each of these affecting vessels of different size ranges (Figure 3).

First, *atherosclerosis* (analogous to a similar process in large arteries) affects distal vessels of diameter 200-800 mm with atheromatous microplaques causing microocclusions. Microocclusions can occur within proximal perforating arteries (microatheroma), at their origin (junctional atheroma) or in the parent artery on the circle of Willis (mural atheroma). These lesions may cause the *lacunae* originally described by Fisher (Ferrer et al., 2008).

Second, *lipohyalinosis* (also termed ‘complex’ small vessel disease) occurs in small, long, scarcely branching arteries of diameter 40-300 mm (Lammie, 2000; Fisher, 1972). It causes fibrinoid deposition in the walls of branches arising directly from large vessels e.g. the lenticulo-striate perforating branches of the middle cerebral artery, the thalamo-perforating branches of the proximal posterior cerebral artery, the perforating branches of the basilar artery and the vessels in the periventricular white matter (Warlow et al., 2008).

Lipohyalinosis is caused by the proliferation of smooth muscle cells from the tunica media, with effusion of plasma proteins through damaged endothelium leading to thickening of the basal lamina and deposition of fibro-hyaline material (Brandner, 2011). In addition, the perivascular space is widened and the surrounding white matter appears gliotic. Finally fibroblasts replace smooth muscle cells in the vascular wall and collagen is deposited resulting in unequal resistance to pressure and irregular lumen diameter (Lammie, 2002; Warlow et al., 2008). These vessel lesions may also be associated with lacunar infarcts.

Third, *arteriolosclerosis* (also referred to as 'simple' small vessel disease) affects vessels of diameter 50-150 mm and causes hyaline thickening of vessel walls and concentric fibrohyalinosis (arteriosclerosis). It occurs in the brain but has been observed in other organs (e.g. kidneys, retinas). This form of microangiopathy is strongly associated with ageing, diabetes and hypertension, and the microvascular changes of arteriolosclerosis are likely the cause of WML (leukoaraiosis) in the elderly (Fisher, 1979; Takebayashi and Kaneko, 1983; Furuta et al., 1991). The pathogenic events start with decreased cerebral blood flow which induces the accumulation of fibrous collagen in the microvascular walls, including those within the white matter. Massive collagen deposits accumulate within the basement membrane and cause narrowing of the lumen and thickening of the microvascular walls (Brandner, 2011).

Fourth, *microaneurysms* are lesions resulting from the destruction of the elastic interna of smooth muscle cells by hyaline connective material. They occur at branching sites in vessels of 100-300 mm diameter (Spangler et al., 1994). Their rupture causes small globular haemorrhages which may correspond to CMBs. After rupture, repair processes involving thrombosis and fibrosis occur, resulting in the creation of fibrocollagenous spheres.

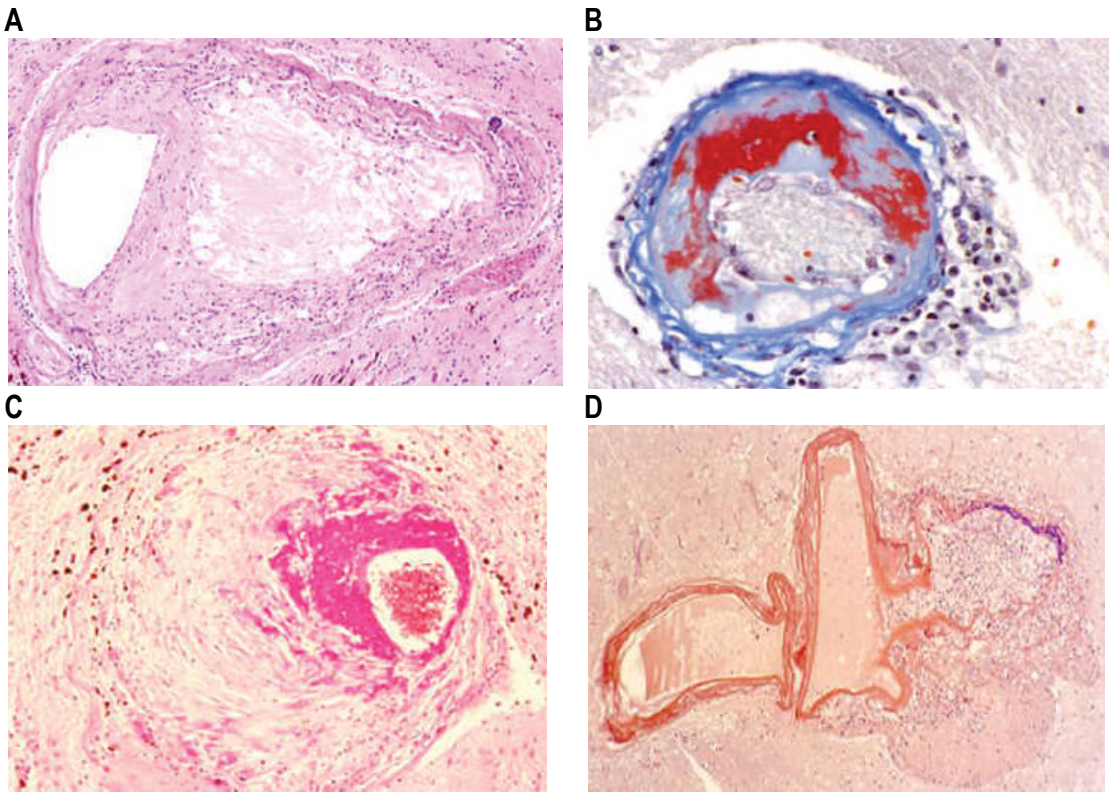


Figure 3. Pathological features of age-related and hypertension-related small vessel disease revealed by haematoxylin-eosin staining

A. Microatheroma caused by atherosclerosis (basal ganglia x20) **B**. Lipohyalinosis (basal ganglia x100). **C**. Arteriolosclerosis with fibrinoid necrosis (pons x20) **D**. Microaneurysm in the right thalamus of a 70-year-old hypertensive patient who died after developing a massive ICH in the left thalamus. Fibrinoid necrosis is visible in the aneurysmal wall.
Adapted from Pantoni, 2010

Finally, *lacunes* are small cavities in the deep brain parenchyma, adjacent to abnormal small vessels. Three types of lacunae have been recognised on histological specimens (Poirier and Derouesne, 1985) (Figure 4). Type 1 lacunae are small old infarcts typically occurring in the putamen, caudate, thalamus, pons, internal capsule and hemispheric white matter. They have been further subclassified into type 1a and 1b. Type 1a consists of small cavities containing small vessels and scattered astrocytes while type 1b are incomplete lacunar infarcts possibly resulting from transient or less severe ischaemia, with loss of selective cellular elements and relative preservation of astrocytes causing patchy astrogliosis (Brandner, 2011). Type 2 lacunes are lacunar cavities filled with numerous haemosiderin-laden macrophages, assumed to have resulted from the re-absorption of small haemorrhages. They are therefore highly relevant to CMBs, the main focus of this work. They may result from haemorrhages within small lacunar infarcts or from primary small haemorrhages. Type 3 lacunes are dilated perivascular spaces (enlarged Virchow-

Robin space) which have attracted increasing attention as a potential neuroimaging marker of small vessel disease (see 1.6.5).

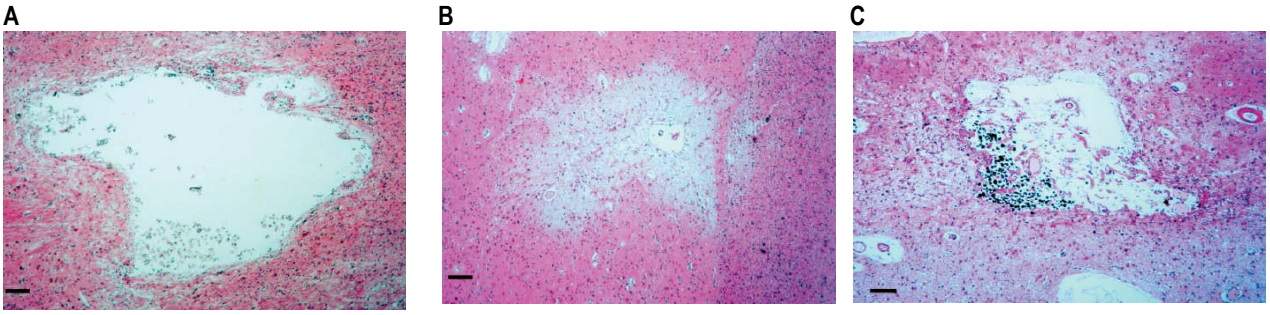


Figure 4. Histological classification of lacunes using haematoxylin-eosin staining

A. Type 1a. Small, old, deep infarct consisting of a small cavity containing occasional small vessels and scattered macrophages. **B**. Type 1b. Incomplete infarct, comprising an area of perivascular rarefaction with loss of all neurones and some oligodendroglia, with patchy astrogliosis. **C.** Type 2. Presumed small deep haemorrhage, in which the cavity contains numerous haemosiderin-laden macrophages. The lesion is surrounded by an area of incomplete infarction.

From Lammie, 2002.

1.3.2 Cerebral amyloid angiopathy

CAA is characterised by the progressive accumulation of congophilic immunoreactive β amyloid protein in the walls of small and medium-sized (10 to 500 μm) vessels located in the cortex and the leptomeningeal space (Corsellis and Brierley, 1954; Pantelakis, 1954; Surbeck, 1961; Jellinger, 1977; Kemper, 1994). The process is also called congophilic angiopathy, which refers to the Congo red stain classically used to demonstrate the twisted β-pleated sheet fibrils of the amyloid substance, which appear with a characteristic 'apple green' birefringence on histological sections under polarised light (Chao et al., 2006; Pantelakis, 1954; Richardson, 1985). An example in cerebral cortical vessels is shown in Figure 5. The affected vessels are predominantly arteries and arterioles, to a lesser extent capillaries, and very rarely venules (Attems et al., 2011). Intimal changes include fibrous thickening, onion skin-like changes, and hyaline changes; an example in leptomeningeal vessels is shown in Figure 6.

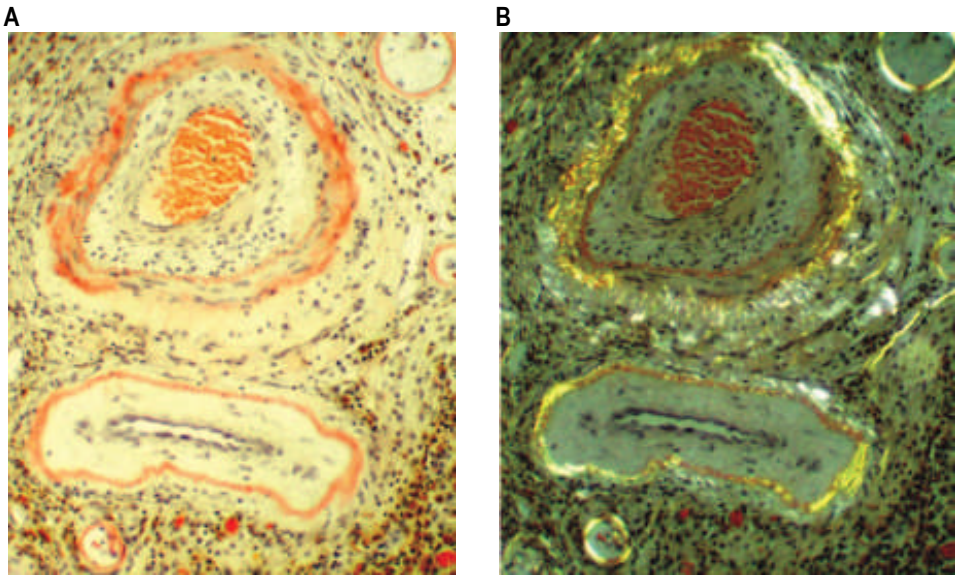


Figure 5. Histopathological appearance of β-amyloid deposition in cerebral cortical vessels.

A. Congo red stain shows highlighted Aβ deposits along the vessel walls. **B**. Congo red stain obtained with polarised light shows the classic yellow-green birefringence of the Aβ deposits. From Chao, 2006

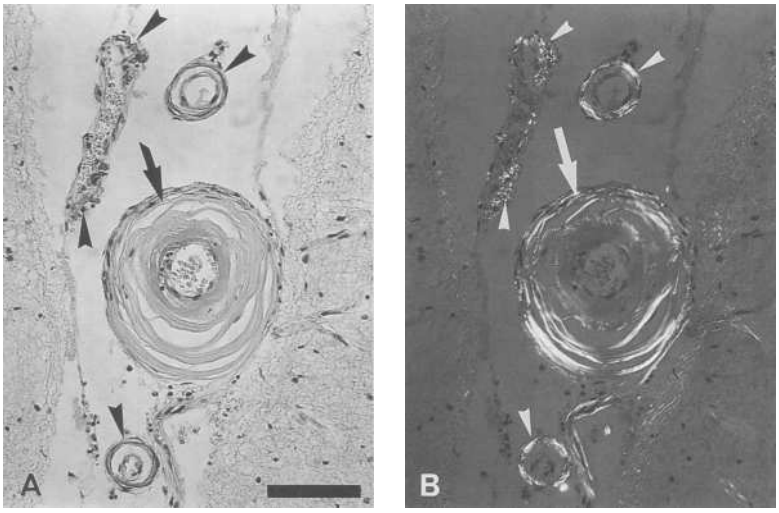


Figure 6. Leptomeningeal vascular changes associated with CAA.

Hyalinous, onion skin-like changes are seen in the intima of the vessel affected (arrows). Congo red staining **(A)** and observation under polarised light **(B)** shows amyloid deposits in the media (arrow heads).
From Yamada et al., 1993

The precise origin of vascular amyloid has not been definitely established, the predominant source appears to be neuronal (Smith and Greenberg, 2009). Amyloid β (Aβ) is generated by the sequential cleavage of the amyloid precursor protein (APP) by β- and γ-secretases (Charidimou et al., 2012b). Mutations occurring in the gene encoding APP account for some

rare familial forms of the disease, including the CAA Dutch type. Mutations in other genes also account for some rare non-Aβ forms of familial CAA. These familial forms of CAA have provided new insights on how mutations in the APP encoding gene contribute to the pathogenesis of the disease. In some families, APP gene mutations render Aβ highly toxic to vessel wall components and more resistant to proteolytic degradation or clearance from the brain (Charidimou et al., 2012b). In the sporadic form of the disease, the mechanisms leading to the deposition of Aβ are not as well understood. Recent studies using transgenic mouse models have demonstrated that Aβ is mainly derived from the neurons. Several potential mechanisms that may initiate and promote Aβ accumulation have been proposed, including: (1) the effects of certain factors that increase the Aβ40:Aβ42 ratio, leading to a shift of Aβ from the brain to the vasculature; and (2) an age-related failure of the mechanisms of Aβ clearance, essentially the perivascular pathways adjacent to cerebral small vessels. The failure of drainage along the perivascular drainage pathways seems to be a key mechanism contributing to CAA pathogenesis (Weller et al., 2009). Recent evidence suggest that perivascular spaces (also referred to as Virchow-Robin spaces) may play a key role in the pathophysiology of CAA. Perivascular drainage may be blocked by the amyloid β deposited in the walls of small arteries, causing dilation of perivascular spaces downstream in the underlying deep white matter, even in regions not directly by CAA pathology (Charidimou et al., 2013c). This hypothesis is supported by the presence of severe enlarged perivascular spaces (EPVS) in the centrum semi-ovale in CAA-related ICH (Charidimou et al., 2013c). Certain genetic factors have been identified as causing a higher risk of CAA and the most important factor is the ApoEε4 allele, which influences CAA risk and age of onset (Verghese et al., 2011). Its effects on Aβ include a shift towards the vasculature and reduced clearance. For further information on the complex pathogenesis of CAA, there is an excellent review article by (Charidimou et al., 2012b).

The reason for the anatomical predilection of Aβ deposition in the cortex and leptomeninges is unknown (Jellinger, 1977; Vinters, 1987). In a pathological series of 68 patients with CAA free of ICH, congophilic vessels were found within the cortex in 75.7%, in the leptomeninges alone in 18.6%, and in the cortex alone in 5.7% (Vonsattel et al., 1991). The cerebral cortex is constantly affected, with the basal ganglia and the thalamus slightly affected in some cases. The brainstem, dentate nucleus, and white matter are usually spared. Furthermore, the CAA process mostly involves the parieto-occipital regions. In a pathological series of 17 brain specimens collected from patients with ICH and CAA, the severity of CAA was slightly, but not significantly, more severe in both the parietal and

occipital lobes than in the frontal lobe and was much less severe in the temporal lobe (Vonsattel et al., 1991). Vinters and Gilbert also noted that the most severe degrees of CAA were parieto-occipital (Vinters and Gilbert, 1983). This apparent predilection for the occipital lobes is not well understood but one hypothesis is that the greater tortuosity of occipital small arteries impairs perivascular drainage (Attems et al., 2011).

Three forms of amyloid deposition have been recognised in the central nervous system: the amyloid core of senile plaques, deposits in the arterial and arteriolar walls, and deposits in the capillary and arteriolar walls with extension into the surrounding brain parenchyma (perivascular amyloid deposition). The latter two conditions are not mutually exclusive and together are called amyloid angiopathy. The vascular alterations are progressive and have been staged by Vonsattel into three grades: (1) mild, (2) moderate and (3) severe (Vonsattel et al., 1991). In mild CAA, the vessel wall is intact and amyloid is present in the abluminal aspect of the tunica media only, with amyloid deposition restricted to a congophilic rim around normal or atrophic smooth muscle fibres. The moderate stage is characterised by a degeneration of the media with replacement by amyloid and thickening. Amyloid deposits are segmentally distributed with apparently normal segments of vessel wall between the amyloid deposits and there are no microaneurysms. Isolated cortical vessels show discrete transverse bands of amyloid, 10 to 20 µm wide, separated by 10 to 20 µm wide amyloid-free segments (Vonsattel et al., 1991). The amyloid-containing bands become increasingly closer to one another as vascular bifurcations approach. As the disease progresses, there is pan-mural accumulation of amyloid and the loss of smooth muscle cells (Attems et al., 2011). In severe CAA, isolated cortical vessels show continuous dense deposits of amyloid, especially thick near or at bifurcations (Vonsattel et al., 1991). There is diffuse and extensive amyloid deposition, foci of fragmentation of the walls, the presence of microaneurysms, and at least one focus of perivascular leakage with blood extravasation (Vinters, 1987). Under light microscopy, the deposition of hyaline material in the vessel wall can be seen causing splitting of the internal elastic lamina and resulting in a ‘double barrel’ appearance, typical of advanced stages of CAA (Pantoni, 2010; Yamada et al., 1993). At this stage, about 10% of vessels show discrete microaneurismal dilatations, and the diameter at this level is 50 to 100% greater than elsewhere. These aneurismal dilatations contain fibrin clots, which arise at points of fibrinoid necrosis of the parent vessel. Fibrinoid necrosis corresponds to discrete foci or segments of vascular wall containing eosinophilic material of plasma proteins (Yamada et al., 1993). The exact pathogenesis of fibrinoid necrosis in CAA is unknown. In severe CAA, breaks in the

continuity of the vessel wall may allow the infiltration of the wall with blood plasma, producing focal necrosis of the media ('plasmatic necrosis') and rupture of the vessel (Vonsattel et al., 1991). Cortical vessels with necrotic walls are frequently accompanied by perivascular fresh microhaemorrhages (haemosiderosis with gliosis), indicating that such vascular changes could be the pathology underlying CAA-related ICH (Yamada et al., 1993). These will be described in more detail in Chapter 2.

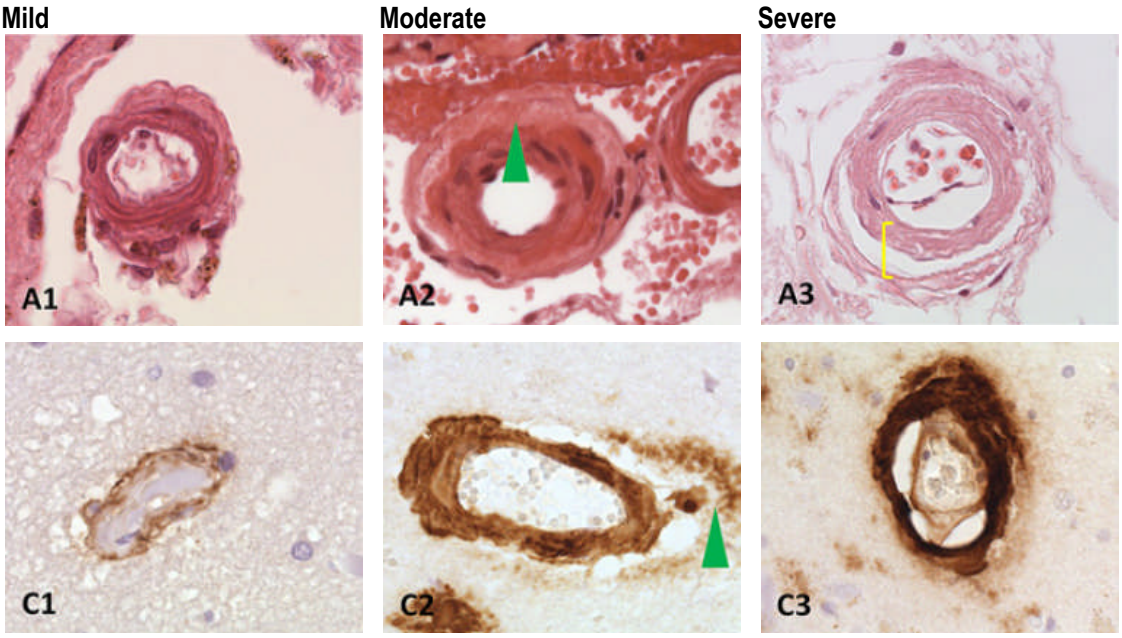


Figure 7. Histopathological changes of mild, moderate and severe CAA.

A1 – A3. Morphological changes of the vessel walls of leptomeningeal arterioles, as revealed by haematoxylin-eosins staining. In mild (A1) and moderate (A2) CAA, only minimal structural changes can be detected. The arrowhead points to amyloid deposition (A2). In advanced CAA (A3), there are significant structural alterations, with visible double barrelling; the bracket represents the detachment of the outer part of the tunica media (A3).
C1 – C3. Immunohistochemical detection of beta-amyloid (Aβ) in cortical arterioles. C2 shows moderate CAA with pan-mural deposition of Aβ along with Aβ deposition in the surrounding brain parenchyma (arrowhead).
From Charidimou et al., 2012a

The alterations to the vessel wall in CAA may occasionally produce stenosis of the lumen resulting in obliteration and cortical infarcts (Vonsattel et al., 1991). However, in most cases the lumen remains patent regardless of the severity of the involvement of the wall. The presence of small infarcts in patients with CAA-related ICH will be studied in Chapter 8.

Thal et al. described two types of sporadic CAA: type 1, associated with the existence of capillary Aβ, and type 2, where capillaries are spared (Thal et al., 2002). A genetic component to these two types is likely, as apolipoprotein E (APOE) ε4 allele is associated with CAA type 1 while APOE ε2 is associated with CAA type 2. The location of Aβ deposits varies within capillaries and larger vessels; in the former, deposits are located at the outer basement membrane, whereas in the latter, they occur in the media near smooth muscle cells.

1.4 Imaging spectrum of small vessel disease

In neuroimaging the term small vessel disease is often used to describe the radiological features (in the brain parenchyma) of the underlying pathological process, appearing on MRI as recent small subcortical infarcts, lacunes, white matter hyperintensities, perivascular spaces, CMBs and brain atrophy (Pantoni, 2010). In this thesis, description of the common MRI features will be based on the recent STRIVE standards for neuroimaging in small vessel disease, an international collaboration aiming at providing Standards for ReportIng Vascular changes on Neuroimaging (Wardlaw et al., 2013). These standards are expected to reliably classify most manifestations of small vessel disease seen on neuroimaging. Lesions with greatest relevance to our work are CMBs, lacunes and white matter hyperintensities of presumed vascular origin. Their distinction is theoretical as the occlusion of microvessels is likely to be involved in both processes and in many patients the two conditions coexist. Lacunes and white matter hyperintensities are often found together, but in some patients one type of imaging appearance may predominate. They will be used in our studies as markers of the presence and severity of small vessel disease.

Before detailing the different imaging features associated with small vessel disease, the important question of how well imaging findings correlate with the underlying pathological processes need to be addressed. Several imaging-pathological correlation studies have examined the neuropathological underpinnings of MRI markers of small vessel disease in post-mortem brains. While in previous studies post-mortem MRI of white matter lesions was less sensitive than pathology, more recent ones show that post-mortem MRI is a valid tool for the assessment of subcortical pathologies (McAleese et al., 2012). McAleese and co-authors demonstrated for the first time that T2-weighted MRI performed on the whole fixed post-mortem brain provides an assessment of small vessel pathology that is comparable to an extensive histological assessment of the entire white matter dissected at 7 mm intervals. Moreover, post-mortem MRI was more accurate than routine histological assessment in frontal, parietal and occipital lobes (McAleese et al., 2012). MRI had lower sensitivity for the evaluation of small vessel pathology in the deep grey matter and in the temporal lobes, likely due to MRI scoring methods causing some small lesions (below 5 mm) to be scored in histological assessment and not in MRI assessment, and possibly to the small volume of temporal lobes. Therefore at least in post-mortem brains, MRI is a valuable technique for translating pathological findings to the clinical setting. The sensitivity of post-mortem MRI for WML was evaluated at 86% (range 79-93%) and specificity at 80% (range

72-88%) (Bronge et al., 2002; Fernando et al., 2004). A recent systematic review of the histopathological correlations of SVD detected on MRI was recently published and showed the large heterogeneity of pathological substrates (Gouw et al., 2011). The sensitivity of MRI for the detection of small vessel pathology *in vivo* remains to be established.

Common imaging features of small vessel disease according to the definitions and imaging standards of the STRIVE collaboration are presented below. A summary of the main lesions related to small vessel disease is provided in Table 3 at the end of this section.

1.4.1 Recent small subcortical infarcts

Recent small subcortical infarcts cause about 25% of all ischaemic strokes (ISs) (Wardlaw et al., 2013) (Figure 8), typically with a clinical 'lacunar syndrome' (see section 1.6.1 below on ischaemic manifestations). They are not always symptomatic in which case they are referred to as silent cerebral infarcts. By contrast, symptomatic lacunar strokes are not always accompanied by visible small subcortical infarcts suggesting that MRI sensitivity is suboptimal to detect these lesions. Recent small subcortical infarcts can evolve in different ways; some develop into a lacunar cavity while others will become a hyperintensity without apparent cavitation or, less commonly, will completely disappear. Rates of cavitation range in studies: from 28% (Potter et al., 2010) to 94% (Moreau et al., 2012). On MRI sequences, their size can be up to 20 mm on axial sections, above the usual size limit (15 mm) for lacunes of presumed vascular origin (Wardlaw et al., 2013). On coronal or sagittal sections, it is not unusual that recent small subcortical infarcts exceed the 20 mm long mark. The pathogenesis of these infarcts is unclear, but thought to be due associated with occlusion of a small perforating artery.

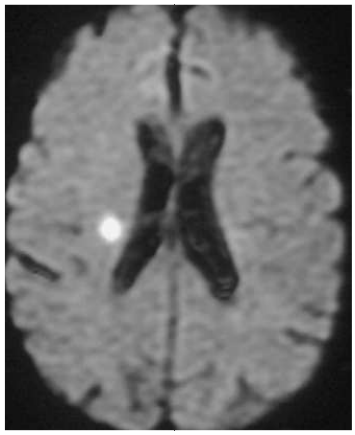


Figure 8. Recent small subcortical infarct.

The lesion is visible in the right centrum semi-ovale on diffusion-weighted imaging (DWI) MRI in the axial plane.

Proposed definitions for the diagnosis of such lesion are that (1) imaging features and clinical symptoms should be consistent with a lesion occurring within the previous few weeks (period including the hyperacute and acute stages), and (2) in the territory of one perforating arteriole, and (3) the lesion should be less than 20 mm in its maximum diameter in the axial plane. Lesions exceeding that size likely result from the occlusion of several penetrating arteries and should be classified as striatocapsular infarcts (Wardlaw et al., 2013). In contrast with lacunes of presumed vascular origin, recent small subcortical infarcts have no lower size limit because they can be distinguished from perivascular spaces using DWI MRI.

1.4.2 Lacune of presumed vascular origin

This terminology proposed by the STRIVE international group allows some uncertainty about the ischaemic or haemorrhagic origin of the lesion and aims at differentiating lacunes from presumed vascular origin from other small brain cavities. Some cavitated small infarcts might be caused by non-small vessel disease mechanisms, such as emboli from atherosclerotic plaques in the carotid arteries or aortic arch (Wardlaw, 2005). According to the recent STRIVE standards, lacunes of presumed vascular origin are round or ovoid, subcortical, fluid filled (similar signal as Cerebrospinal Fluid [CSF]) cavity, of between 3 mm and about 15 mm in diameter, consistent with a previous acute small deep brain infarct or haemorrhage in the territory of one perforating arteriole (Wardlaw et al., 2013) (Figure 9). On fluid-attenuated inversion recovery (FLAIR) sequence, lacunes of presumed vascular origin have a characteristic CSF-filled core surrounded by a rim of hypertensity (Brown et al., 1988; Offenbacher et al., 1996). Variations exist, e.g. absent rim or no evidence of central cavity fluid (the lesion appears entirely hyperintense).

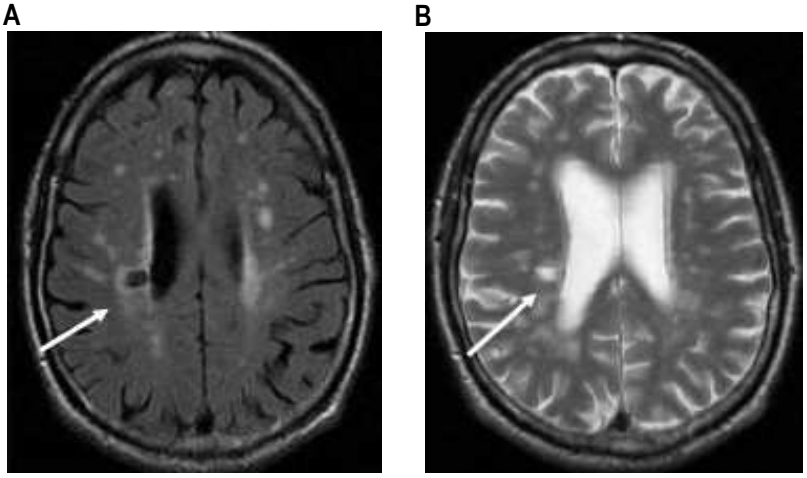


Figure 9. Imaging characteristics of a lacune of presumed vascular origin on MRI

A. Typical appearance of a lacune on FLAIR imaging, consisting of a hypointense CSF-containing hole (white arrow). **B**. Corresponding T2-weighted MRI sequence showing a hyperintense area (white arrow) surrounded by other smaller white matter lesions.
From Wardlaw, 2010 .

Of note, perivascular spaces entering an area white matter hyperintensity may mimic lacunes of presumed vascular origin on FLAIR sequences. Perivascular spaces can be distinguished from lacunes based on their very small size, below the 3 mm diameter cut-off (Longstreth et al., 1998; Vermeer et al., 2003b). The maximum diameter of lacunes of vascular origin is 15 mm, smaller than that of recent small subcortical infarcts, because of tissue loss in old lesions and swelling in new lesions.

1.4.3 White matter hyperintensity of presumed vascular origin

WML are seen on T2-weighted MRI as bilateral, mostly symmetrical hyperintensities (Figure 10). They can appear isointense or hypointense but not as hypointense as CSF on T1-weighted MRI, depending on the sequence parameters and severity of the pathological changes. They can also occur in the subcortical grey matter structures (i.e. basal ganglia), and in the brainstem, where they should be termed subcortical hyperintensities (Wardlaw et al., 2013). Whether periventricular and deep white matter hyperintensities are part of the same process and share similar or different pathogenesis, risk factors and clinical consequences is a matter of debate. The term leukoaraiosis first coined by Vladimir Hachinski describes the radiological appearance of periventricular attenuation on CT and was later transposed to appearances on T1 weighted, T2 weighted and FLAIR MRI sequences. The STRIVE collaboration proposed the term white matter hyperintensity of presumed vascular origin to exclude WML of other causes (e.g. leukodystrophies, multiple sclerosis, metabolic or inflammatory disorders). When using CT, the term white matter hypoattenuation or white matter hypodensities can be used because of the appearance of the lesions on CT.

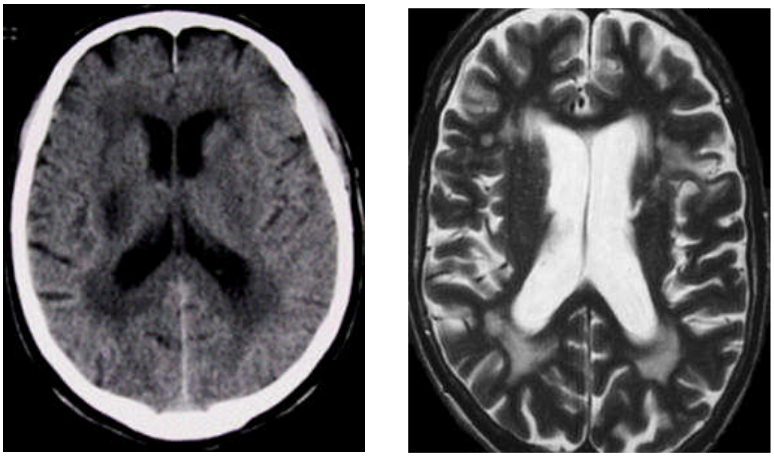


Figure 10. Radiological characteristics of white matter hyperintensity of presumed vascular origin

A. Periventricular lesions on CT are termed white matter hypoattenuation or white matter hypodensities **B.** T2-weighted MRI showing hyperintensities compatible with white matter hyperintensities of presumed vascular origin

White matter hyperintensities of presumed vascular origin are increasingly common with advancing age and strongly associated with cerebrovascular disease and vascular risk factors. Their severity varies considerably across individuals from a single focus (mild) to extensive involvement (focal areas to large confluent areas) of the subcortical white matter (Inzitari et al., 2009) (Figure 11). Accurate measurement of WML burden at presentation and progression over time is of crucial importance for epidemiological studies to determine the associations between WML, cognitive and clinical data; their causes and the effects of new treatments in randomised trials (Scheltens et al., 1998; Valdés Hernández et al., 2013). Contradictory results between studies exploring the frequency, clinical significance and risk factors of WML result in part from difference in study methods and heterogeneity of patient populations, but also in differences in the methods used to evaluate the degree of white matter damage. For example, the correlation between white matter hyperintensities and cognition has been inconsistent across studies, possibly reflecting heterogeneity of the quantifying methods deterring their application (Gao et al., 2011). Among the many methods to measure WML developed over the recent decades, visual (qualitative) rating scales and computational volume measurement (quantitative) methods are used in routine clinical practice and research studies.

Here, I will develop three scales of white matter hyperintensities on MRI scans that appear most frequently in the literature: the scale by Fazekas, the age related white matter scale

by Wahlund and the Scheltens scale. Fazekas and colleagues proposed 2 different scores for periventricular and white matter lesions on T2-weighted MRI, rated on a 3-point scale: periventricular score = absent (grade 0), caps or pencil-thin lining (grade 1), smooth halo (grade 2), irregular lesions extending into the deep white matter (grade 3); and white matter score: absence (grade 0), punctuate foci (grade 1), beginning of confluence of foci (grade 2), or large confluent areas (grade 3) (Fazekas et al., 1987). Reliability was tested by Leys et al and found to be poor (Leys et al., 1990). The rating scale is easy to apply in most cases, even on poor-quality MRI scans. The second scale is the scale by Wahlund et al, and is the one used in this thesis (Wahlund, 2001). The degree of white matter changes and periventricular lesions was jointly rated on a 4-point scale, with scores ranging from 0 to 3. No anatomical information is provided. Examples of the different degrees of severity based on this scale are illustrated in Figure 11. The Scheltens scale is a 'Fazekas plus' score that provides 4 scores in a semiquantitative way: periventricular hyperintensities score (0 to 6); white matter hyperintensities score (0 to 24), basal ganglia and infratentorial hyperintensity scores (both 0 to 24). As number and size of hyperintensities are taken into account, the score provides a surface-based volume score, and the detail of individual regions adds extra anatomical information. The scale had good reliability for assessment of white matter hyperintensities (Scheltens et al., 1993).

Table 2. Example of rating scale for age-related white matter lesions MRI and CT

From Wahlund et al., 2001

White matter lesions	
0	No lesions (including symmetrical, well-defined caps or bands)
1	Focal lesions
2	Beginning confluence of lesions
3	Diffuse involvement of entire region (with/without involvement of U-fibres)
Basal ganglia	
0	No lesions
1	1 focal lesion (≥ 5 mm)
2	≥ 1 focal lesion
3	Confluent lesions

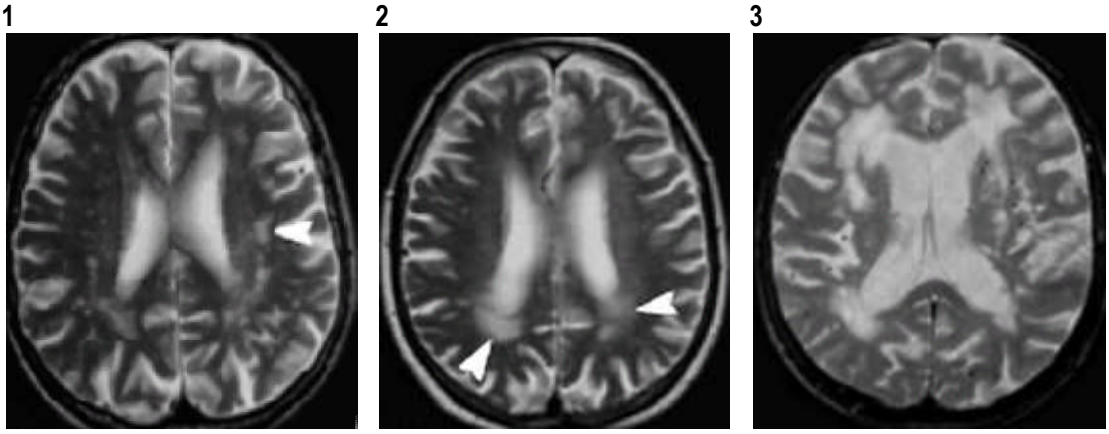


Figure 11. Illustration of different severity of white matter hyperintensities of presumed vascular origin on axial T2-weighted MRI sequences.

(1) Focal lesions **(2)** starting confluence of lesions and **(3)** diffuse involvement of the entire region. These images correspond to Wahlund scale grades 1, 2 and 3 respectively

Adapted from Wahlund et al., 2001.

As shown above, these visual scales vary greatly in the degree of complexity in regard to morphology, size, number and anatomical distribution of WML. There have been four previous comparisons of visual rating scales and quantitative WML volume measurements showing inconsistent results (Valdés Hernández et al. 2013). Nevertheless, visual and volumetric methods of WML assessment were highly correlated in a recent study comparing the most widely used and well-validated scale (Fazekas scale) with a volumetric method using a validated a validated image-processing method (Valdés Hernández et al., 2013). WML rating scores and volumes provided near-equivalent estimates of WML burden. Therefore the authors suggested that either method can be used depending on the particular characteristics of the population or the circumstances of the data collection, taking advantage of the strengths of each technique. Both qualitative and quantitative approaches have advantages and disadvantages. Visual rating scales are relatively quicker and easier to perform with relatively good reliability on scans from varied modalities or qualities, and perhaps practically easier to use in clinical and research settings. Computer-assisted quantitative volumetric measures are more objective and precise than the rating scales, but are technically more demanding and time-consuming (Gao et al., 2011). In research studies, a combination of qualitative and quantitative methods is acceptable if appropriate statistics are used. However, because stroke lesions are not separated from total WML volume in volumetric methods, qualitative ratings should be used in studies of WML in patients with stroke, and if volumes are measured, strokes will have to be manually separated from stroke lesions (Valdés Hernández et al., 2013). In this thesis, when severity

of WML was measured, we chose to use the scale developed by Wahlund as a qualitative method in agreement with these findings. The scale has been validated against volumetric measurement (van Straaten et al., 2006) and has been widely used in recent studies. It has provided similar scores on CT and MRI in various regions, with a fair intra-rater reliability (κ = 0.48) (Wahlund et al., 2001).

1.4.4 Perivascular space

Perivascular spaces, also known as Virchow-Robin spaces, type 3 lacune - or *état criblé* when extensive and located predominantly in the basal ganglia - are visible on T2-weighted and sometimes on T1-weighted MRI in the basal ganglia and hemispheric white matter. They can also be seen in the midbrain but rarely in the cerebellum. They have been recognised pathologically for many years and correspond to interstitial fluid filled spaces that follow the typical course of a arteries, arterioles, veins and venules as they go through grey or white matter. They are believed to function as the brain drainage system (Marin-Padilla and Knopman, 2011; Ozturk and Aydingoz, 2002) and may play a key, previously underestimated role in the pathogenesis of CAA (Charidimou et al., 2013c). They are commonly microscopic, but larger spaces become increasingly apparent with increasing age, especially when located at the basis of the brain (Wardlaw et al., 2013). On MRI, they can be distinguished from lacunes because they are usually smaller (< 3 mm) and have an isointense appearance with the CSF on all sequences (Hirabuki et al., 1994). They can appear linear, when imaged parallel to the course of the vessel, and round or ovoid, when imaged perpendicular. General enlargement of perivascular spaces is associated with ageing and hypertension (Bokura et al., 1998), and with other markers of small vessel disease such as white matter hyperintensities (Doubal et al., 2010)and lacunes (Kwee and Kwee, 2007). As such, they are believed to be another expression of small vessel disease in the brain (Doubal et al., 2010). However, the clinical consequences of increased size of perivascular spaces are not fully understood, and their detection largely dependent on imaging methods, therefore the term ‘enlarged’ or ‘dilated’ should be avoided (Wardlaw et al., 2013). Their systematic study in patients with spontaneous ICH suggested that the distribution of severe large perivascular spaces reflects the underlying arteriopathy type (Charidimou et al., 2013c). Severe perivascular spaces in the centrum semi-ovale are more often severe in patients with strictly lobar ICH (ie, probable or possible CAA) while in the basal ganglia they are associated with markers of hypertensive arteriopathy including WML

and deep CMBs (Charidimou et al., 2013c). A predilection of numerous visible perivascular spaces in the centrum semi-ovale rather than the basal ganglia may therefore be an indicator of CAA.

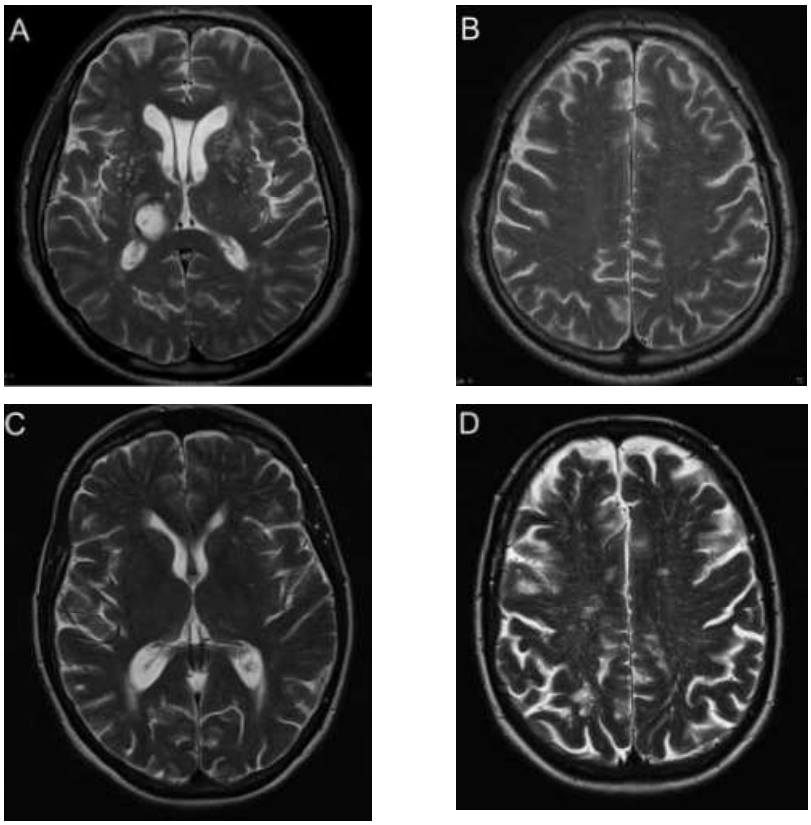


Figure 12. Axial T2-weighted MRI.

A. and **B**. Dot-like hyperintensities characteristic of perivascular spaces in the basal ganglia in a patient with spontaneous deep intracerebral haemorrhage (ICH). **C**. and **D**. Linear hyperintensities typical of perivascular spaces in the centrum semiovale in a patient with CAA-related ICH. Note that deep brain regions (e.g. basal ganglia) are not affected (D).
Adapted from Charidimou et al., 2013

1.4.5 Cerebral microbleeds

CMBs are small hypointense lesions that are visible on paramagnetic sensitive MR sequences such as T2* weighted gradient recalled echo (GRE) or susceptibility weighted sequences (Figure 13). They are generally not seen on CT, or on FLAIR, T1 weighted or T2 weighted sequences. They are most commonly located in the deep grey or white matter in the cerebral hemispheres, brainstem and cerebellum, and in the cortico-subcortical junction. Imaging pathology correlation studies suggest that they correspond to haemosiderin laden macrophages in perivascular tissue, hypothesized to result from

vascular leakage from abnormal, fragile small vessels (De Reuck et al., 2011; Fazekas et al., 1999; Shoamanesh et al., 2011). They can be differentiated from an old small deep spontaneous ICH because ICH tend to be larger, irregular with a cystic cavity, and are visible on T1 weighted, T2 weighted or FLAIR sequences (Wardlaw et al., 2013). Recommendations for standardised imaging and reporting of CMBs have been published by the Microbleed Study Group (Greenberg et al., 2009b). CMBs will be covered extensively in the next chapters: chapter 2 is dedicated to the understanding of the pathophysiology, histopathology and detection of CMBs and Chapter 3 provides an overview of the clinical implications of CMBs.

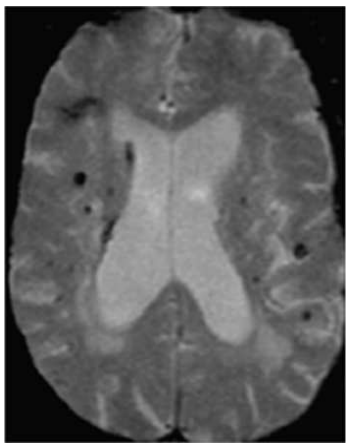


Figure 13. Multiple CMBs in the cortex and subcortex of a patient with probable CAA, as shown on a gradient-recalled echo T2* MRI sequence

1.4.6 Brain atrophy

Brain atrophy is defined as a reduced brain volume that is not related to a specific macroscopic focal injury such as trauma or infarction (Wardlaw et al., 2013). Tissue loss is assumed from the enlargement of peripheral (sulcal) and central (ventricular) CSF spaces in relation to intracranial volume and other measures. It can be general or focal, symmetrical or asymmetrical, or tissue selective (e.g. for the white matter). Neuropathological substrates of atrophy are neuronal loss, cortical thinning, subcortical vascular pathology, arteriolosclerosis, venous collagenosis and secondary neurodegenerative changes (Wardlaw et al., 2013). An association between the presence and severity of small vessel disease and brain atrophy has been reported in many studies (Appelman et al., 2009; Aribisala et al., 2013). Therefore, brain atrophy is a measure that should be included in imaging studies assessing the effects of the vascular burden in the brain, and it is thought

to mediate, at least partially, the effects of vascular lesions on cognition (Carmichael et al., 2010; Mungas et al., 2001).

1.4.7 Other haemorrhagic brain lesions

Spontaneous ICH can be a manifestation of small vessel disease and a recommended term is spontaneous ICH presumably due to small vessel disease (Wardlaw et al., 2013). These haemorrhages are non-lobar haemorrhages, in contrast with lobar haemorrhages thought to be caused by CAA. The Boston criteria for CAA should be used in assigning the likelihood of underlying CAA in patients with lobar ICHs (see next section) (Knudsen et al., 2001). Treatable underlying causes of haemorrhages ('secondary' haemorrhages') have to be identified before a haemorrhage can be classified as spontaneous. Detection of an underlying arteriovenous malformation, aneurysm, dural arteriovenous fistula or intracranial venous thrombosis is important because timely treatment can prevent recurrent ICH. Furthermore, delayed MRI may help identify an underlying tumour or cavernous malformation. The outcome after spontaneous ICH seems to be worse than after a bleed secondary to an arteriovenous malformation (van Beijnum et al., 2009), which justifies thorough investigation for all patients. Investigations into common causes of secondary ICH include taking further history from patients and others (about trauma or drug use), routine laboratory tests to search for vasculitis, cirrhosis or neoplasms, coagulation studies, blood cultures, toxicology, CSF analysis, and imaging studies including CT, CT angiography and venography, MRI, MR angiography and venography, and cerebral catheter angiography (Josephson et al., 2010). However, despite the wide variety of technologies for investigating ICH, there is a shortage of evidence and lack of consensus about who, when and how to further investigate for a cause underlying ICH (Josephson et al., 2010). From a systematic review and structured survey of clinicians in three different countries, younger patient age was the strongest influence on the decision to further investigate ICH (Cordonnier et al., 2010). Further studies of the diagnostic accuracy of non-invasive investigations are required, but it is unlikely these techniques will replace the intra-arterial digital subtraction angiography. Until then, clinicians will be guided by the patient's clinical characteristics and condition and their local healthcare resources.
Superficial cortical siderosis is the radiological correlate of the presence of chronic blood products (haemosiderin) in the superficial layers of the cerebral cortex. It is visible on T2* weighted GRE and other blood sensitive sequences as a linear hypointensity over the cortex and should not be confused with the petechial cortical haemorrhagic transformation

of an infarct. It is likely the radiological sequelae of repetitive bleeding in the subarachnoid spaces or the chronic lesions following acute convexity SAH. Superficial cortical siderosis has been included as an additional haemorrhagic lesion in the revised criteria for the diagnosis of CAA, with potential diagnostic significance equivalent to lobar ICH (Linn et al., 2010). It can be detected in up to 50% of patients with a clinico-radiological diagnosis of CAA (Linn, Neurology 2010) and may be associated with transient neurological manifestations (Charidimou et al., 2012a; Roch et al., 2005).

Convexity subarachnoid haemorrhage (SAH) results from the extension of a lobar haemorrhage to the subarachnoid space over the cortical surface. The bleeding is localised to up to several adjacent sulci and is frequently associated with CAA. In a report of 29 patients with atraumatic convexal SAH from a consecutive series of patients with SAH, 9 of the 13 (69%) patients aged over 60 years had signs of superficial siderosis, white matter hyperintensities and CMBs, all compatible with the diagnosis of CAA (Kumar et al., 2010). Convexity SAH and cortical superficial siderosis are recently recognised imaging correlates of sporadic CAA (Linn et al., 2008) and seem to be characteristic of the disease (Figure 14).

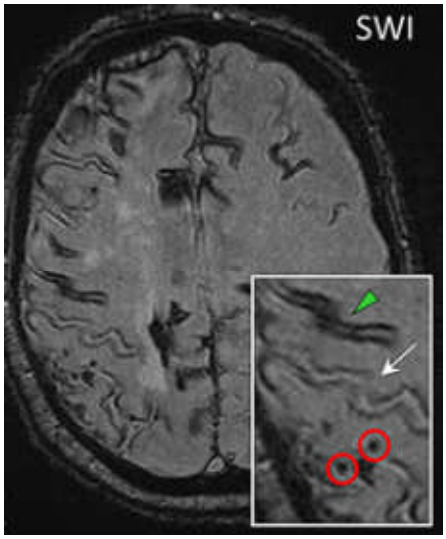
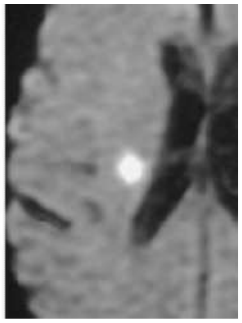
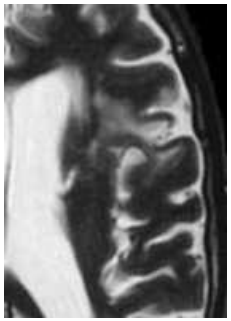
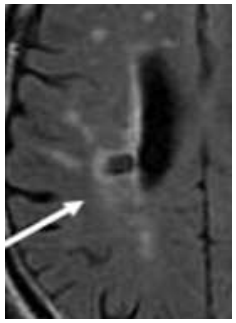
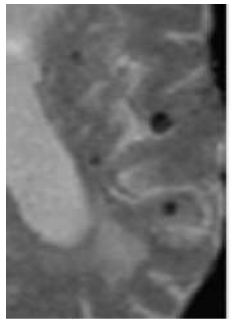


Figure 14. Susceptibility-weighted imaging (SWI) showing the coexistence of convexity SAH, cortical siderosis and CMBs in a patient with CAA.

The inset demonstrates the linear hypointensities in the subarachnoid space suggestive of convexity SAH (green arrowhead) and focal cortical siderosis in the adjacent sulcus (white arrow) surrounded by some CMBs (red circles).

From Charidimou et al., 2012a

Table 3. Main lesions related to small vessel disease according to the definitions and imaging standards of the STRIVE collaboration (adapted from Wardlaw, 2013)

	Recent small subcortical infarct	White matter hyperintensity	Lacune	Perivascular space	Cerebral microbleed
Example image					
Usual diameter	≥ 20 mm	Variable	3-15 mm	≤ 2 mm	≤ 10 mm
Main characteristic	Early identification on DWI	Located in white matter	Usually have hyperintense rim	Most linear without hyperintense rim	Detected on GRE T2* sequence, round or ovoid, blooming effect
Presumed mechanism of formation	Occlusion of a small perforating artery	Likely multifactorial	Healed stage of a small subcortical infarct or small deep haemorrhage	Fluid-filled space following the course of a vessel as it goes through grey or white matter	Likely haemosiderin-laden macrophages in perivascular tissue consistent with vascular leakage of blood cells
MRI findings:					
DWI	↑	↔	↔/(↓)	↔	↔
FLAIR	↑	↑	↓	↓	↔
T2	↑	↑	↑	↑	↔
T1	↓	↔/(↓)	↓	↓	↔
GRE T2*-weighted	↔	↑	↔ (↓ if haemorrhage)	↔	↓↓

↑ Increased signal ; ↓ Decreased signal ; ↔ Iso-intense signal

1.5 Mechanisms of small vessel disease

Clinical CT-correlated studies and pathological studies of the autopsied brain have found that systemic hypertension is closely associated with ischaemic lesions such as lacunes or white matter hyperintensities and with intracerebral haematomas (Fisher, 1972; Cole and Yates, 1967; Cole and Yates, 1968; Fisher, 2003). The same type of hypertensive small artery-disease (i.e. involving parenchymal small arteries and arterioles) can cause either ICHs or ischaemic lesions, depending on the circumstances (Fisher, 1971a). Abnormally high BP is associated with the increased occurrence of lacunes (Cole and Yates, 1968), and favours the development of more extensive white matter hyperintensities (Awad et al., 1986; Almkvist et al., 1992; Breteler, 2000; Wiszniewska et al., 2000; Hirono et al., 2000). Other cardiovascular risk factors associated with white matter hyperintensities and lacunes are diabetes mellitus and smoking (Yamauchi et al., 2002). However, in spite of the recognised risk factors, the pathogenesis of age and hypertension-related small vessel disease remains incompletely understood. It is unclear why some patients develop white matter hyperintensities, whilst others also develop lacunes. Hypertension is the major risk factor for all types of cerebral small vessel disease, but fails to account for all of the risk; some of the risk remains unexplained by conventional risk factors. For example, CAA is generally not considered to be related to conventional risk factors, apart from age. Moreover, small vessel disease occur in normotensive, non-elderly and non-diabetic patients, and the amount of lacunar infarcts is not directly related to the severity of hypertension. Therefore, genetic predisposition has been widely speculated upon.

There is now evidence that genetic factors play an important part in the susceptibility of vessels to damage. For example, a positive family history of stroke is a risk factor for lacunar stroke. In lacunar stroke patients below the age of 65 years, a parental family history of stroke is more frequent in patients with silent cerebral small vessel disease compared with probands without silent small vessel disease, 59% versus 20%, respectively (Knottnerus et al., 2011). The APOE ε4 allele may confer some risk of developing small vessel pathology, as there is evidence that amyloid beta peptide levels are associated with an increased risk for lacunar stroke in carriers of the APOE ε4 allele (van Dijk et al., 2004). APOE ε4 was recently found to have a dose dependent association with sporadic CAA (Rannikmae et al., 2013). Furthermore, APOE ε4 carriers are at increased risk for the development of subcortical WML, if they are also hypertensive (de Leeuw et al., 2004). These observations require further confirmation. Additional genetic factors that may play a

role in the development of small vessel disease and lacunar stroke include homozygosity for the T allele located at position -7351 within the enhancer region of the tissue plasminogen activator gene (Jannes et al., 2004) and the D allele of the angiotensin converting enzyme insertion/deletion genotype (Hassan et al., 2002). There probably are other genes awaiting identification. The existence of inherited conditions characterised by hereditary small vessel arteriopathy also supports the idea of a genetic contribution to small vessel disease. These conditions have been identified in recent years and include Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), Familial Cerebral Amyloid Angiopathy, Hereditary Endotheliopathy with Retinopathy Nephropathy and Stroke (HERNS) and Cerebral Autosomal Recessive Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CARASIL). Likewise, mutations in the genes encoding for some vascular basement membrane components, such as the type IV collagen, have recently been identified as a cause of brain small vessel disease with haemorrhage (Gould et al., 2006; Vahedi et al., 2007). All these conditions are of clinical and research interest as 'pure' forms of cerebral small vessel disease, i.e. non-vascular risk factors related. While all of these conditions affect small vessel and may theoretically manifest as a classic lacunar syndrome, CADASIL is most likely to do so. CADASIL is a single gene disorder responsible for lacunar strokes and severe white matter damage that result from a mutation in the NOTCH 3 gene, which encodes for a transmembrane receptor protein (Joutel et al., 1996; Tournier-Lasserve et al., 1993). NOTCH 3 is predominantly expressed in vascular smooth muscle cells and its protein product, Notch-3, is critical for the structural and functional integrity of small arteries (Viswanathan et al., 2011). Recent research suggests that a mutated gene may convey a non-physiological and deleterious activity to the Notch-3 receptor or may result in a loss of function in the receptor. The condition is associated with a systemic arteriopathy with particularly severe involvement of the cerebral small vessels. The main clinical manifestations include attacks of migraine with aura, mood disturbances, recurrent ISs and progressive cognitive decline (Dichgans et al., 1998; Chabriat et al., 1995). The patients have a combination of small lacunar infarcts, and more diffuse chronic ischaemic changes, seen on imaging as white matter hyperintensities (leukoaraiosis) that is more extensive than normal. MRI features highly suggestive of CADASIL are the confluent involvement of the anterior temporal lobes, which is present in 90% of the patients with CADASIL and is rare in hypertension-related small vessel disease (Markus et al., 2002). Genetic screening is now available in many laboratories worldwide. We will end this section on inherited

conditions or 'pure' forms of small vessel disease by describing Fabry disease, an X-linked lysosomal storage disease with deficient α galactosidase causing endothelial vasculopathy and cerebral ischaemia. Up to 0.5% of cryptogenic strokes in patients aged 18 to 55 are due to Fabry disease and therefore the diagnosis must be considered in all cases of unexplained stroke in young patients (Rolfs et al., 2013). Active research in the diagnosis and treatment of these conditions will help to improve our understanding of cerebral small vessel disease.

The mechanisms leading to parenchymal damage in cerebral small vessel disease are complex and not entirely known, and may result from a combination of genetic and environmental risk factors. A hypothesised cascade combining both mechanisms was proposed by Pantoni (2010), and is shown in Figure 15. Pathophysiological events can lead to haemorrhages, either microhaemorrhages (CMBs) or macrohaemorrhages, and to ischaemia. The clarification of the pathological changes described above in hypertensive arteriopathy and CAA allows a better understanding of the mechanisms leading to ICH. However, the reasons why some vessel ruptures result in major haemorrhages while others result in microhaemorrhages remains unknown. In CAA, it is believed that differences in wall thickness are linked with haemorrhage sizes, with thicker walls possibly being more susceptibole to small extravasations and thus associated with microhaemorrhages (Greenberg et al., 2009a). With regard to ischaemia, the pathogenesis of ischaemic lesions associated with small vessel disease remains hypothetical and several mechanisms have been proposed. Firstly, the vessel lumen restriction leads to a chronic state of hypoperfusion of the white matter, eventually resulting in the degeneration of the myelinated fibres as a consequence of repeated selective oligodendrocyte death (Pantoni, 2010). White matter hyperintensities (leukoaraiosis) identified on neuroimaging is believed to result from this putative mechanism. A second theory is that affected small vessels may develop acute occlusions leading to acute and focal ischaemia, and tissue necrosis. The microatheroma obstructs the vessel wall and may be the cause of lacunar infarcts visible on brain imaging. This mechanism remains speculative due to little supportive pathological data being available. Other mechanisms have been suggested and include: blood-brain barrier disruptions (Wardlaw et al., 2003), endothelial dysfunction and a reduced capacity for endothelial regeneration (Rouhl et al., 2009), oligodendrocyte apoptosis (Brown et al., 2000), and local subclinical inflammation (Rosenberg, 2009; Simpson et al., 2007).

Figure 15. Pathogenesis of brain damage resulting from small vessel disease, based on the hypothesis by Greenberg and colleagues (2009)

From Pantoni et al., 2010

In CAA, the hallmark of the disease is the deposition of Aβ in the vessel walls. As shown in section 1.3.2, Aβ derived from the APP can deposit in the brain parenchyma to form senile plaques or can be carried into the perivascular space via the interstitial fluid to become cerebrovascular deposits (Weller et al., 1998). Deposition within the vessel walls causes injury to the vessel, which can give rise to small infarctions (Kimberly et al., 2009; Soontornniyomkij et al., 2010) or to rupture of the vessel wall with leakage of blood, and formation of small CMBs, microaneurysms or larger symptomatic ICHs. Possible mechanisms to explain small cortical infarctions in CAA will be proposed in Chapter 8. ICHs are of a subcortical or lobar type due to the predilection of Aβ for the cortical and leptomeningeal arteries. The predisposition for haemorrhage likely relates to structural changes in the vessel walls, with the vessels becoming brittle, fragile and prone to

microaneurysm formation and leakage (Mandybur, 1986; Vonsattel et al., 1991). Mild CAA may cause no evident vessel damage, but in increasingly severe CAA, the progressive degeneration of the tunica media with loss of smooth muscle cells, fibrinoid necrosis, microaneurysms and leakage of blood products is responsible for haemorrhages (Mandybur, 1986; Neumann, 1960; Torack, 1975; Okazaki et al., 1979). Risk factors that predispose to ICH in CAA are the severity of CAA and the presence of fibrinoid necrosis (Okazaki et al., 1979; Corsellis and Brierley, 1954; Dubas et al., 1985; Gray et al., 1985; Mandybur, 1986; Regli et al., 1981; Vonsattel et al., 1991; Ferreiro et al., 1989). The prevalence of CAA-related ICH is considerably lower than the overall prevalence of CAA. This suggests that only the most severely affected minority of patients with CAA develop ICH, indirectly supporting a link between CAA severity and ICH.

However, CAA does not necessarily result in ICH. In the study by Vonsattel and colleagues, 11 patients without ICH had severe CAA (Vonsattel et al., 1991). The majority of the brains from patients with CAA were free of haemorrhage (Yamada et al., 1987), and in addition, cerebral haemorrhage in CAA may occur without any evidence of vessel wall necrosis (Julien et al., 1983). It is likely that CAA alone is not sufficient to explain the occurrence of haemorrhage, thereby suggesting that other mechanisms may play a role. Acute trigger factors, e.g. sudden increases in BP or minor trauma, may cause the rupture of these abnormally weak, amyloid-laden vessels (Charidimou et al., 2012b). Other factors may also interact with CAA to cause lobar ICH, e.g. hypertension, associated neurodegenerative pathology, use of anticoagulant drugs. Other mechanisms of brain injury in CAA have been suggested including impaired local regulation of cerebral blood flow (Smith and Greenberg, 2009) and impaired neurovascular unit function (Iadecola, 2004). Similarly to hypertensive arteriopathy, blood-brain barrier disruption and active inflammation could also contribute (Attems et al., 2011; Smith and Greenberg, 2009).

Several genetic polymorphisms have been associated with CAA and should contribute in increasing our understanding of the mechanisms leading to CAA. Polymorphisms in the APOE gene have been the most studied, but it is only recently that APOE polymorphisms have been studied in histopathologically confirmed CAA (Rannikmae et al., 2013). An association between APOE and ICH attributed to clinically confirmed CAA is well described in case control studies of CAA-related ICH versus CAA-free controls but such studies were not able to distinguish a genetic association with CAA from an association with ICH. APOE *in vitro* influences Aβ conformation, fibril formation and toxicity (Lambert et al., 1998) and in

mouse models contributes *in vivo* to Aβ deposition, toxicity and possibly clearance (Holtzman et al., 2000). The APOE gene on chromosome 19 has 3 common alleles – ε2, ε3 and ε4 – giving rise to 6 different genotypes: ε2/2; ε2/3; ε2/4; ε3/3 (the wild type, occurring in just under two-thirds of people in most populations (Menzel et al., 1983), ε3/4 and ε4/4. Alleles recognised for an implication in CAA pathogenesis are APOE ε4 and APOE ε2; it is believed that the former enhances amyloid deposition in cerebral blood vessel walls (possibly by increasing aggregation and/or impairing clearance) while the latter promotes haemorrhage from amyloid laden blood vessels by increasing specific CAA related vasculopathic changes including fibrinoid necrosis (Greenberg et al., 1998; Holtzman et al., 2000; McCarron and Nicoll, 2000; McCarron et al., 1999). A recent meta-analysis of published studies of the associations between any genetic polymorphism and histopathologically confirmed CAA showed a significant association of APOE ε4 with CAA (Rannikmae et al., 2013); the odds of having CAA more than doubled for ε4+ genotypes independently of ethnicity and dementia status. More so, the risk of having CAA increased with increasing dose of the ε4 allele (ε4-- versus ε4+- versus ε4++). There was no significant association between APOE ε2 and CAA. Preliminary evidence of other polymorphisms was also reported but results should be replicated in other studies (Rannikmae et al., 2013). It is likely that both CAA and AD share this 'mechanistic' effect of APOE ε4. In Alzheimer's, neurodegeneration may arise from additional causes, ie, via specific neurotoxic effects of APOE, independent of interactions with Aβ (Verghese et al., 2011).

With regards to ischaemia, white matter hyperintensities (leukoaraiosis) of presumed vascular origin are very common in patients with CAA. The pathogenesis of leukoaraiosis in CAA probably involves chronic hypoperfusion of the vulnerable periventricular white matter and disruption of the blood-brain barrier due to amyloid in the overlying cortical small vessels (Charidimou et al., 2012b). Another possible mechanism is the accumulation of small ischaemic lesions or microinfarcts. Neuropathological studies have also demonstrated the presence of small cortical infarcts in the brains of patients with CAA (Olichney et al., 1995; Cadavid et al., 2000; Haglund et al., 2006b). These can be detected *in vivo* using MRI, an imaging technique very sensitive to acute ischaemia. This has potential implications for cognitive impairment and disability in patients with CAA and leukoaraiosis. We conducted a neuroimaging study in patients with CAA-related ICH and other types of spontaneous ICH to confirm this intriguing phenomenon *in vivo* (see Chapter 8). Possible mechanisms to explain the presence of ischaemic lesions in CAA will be suggested.

1.6 Clinical spectrum of cerebral small vessel disease

Cerebral small vessel disease can present as a stroke or cognitive decline, or can have few or no symptoms (Wardlaw et al., 2013). Small vessel disease frequently coexists with neurodegenerative disease and can exacerbate cognitive deficits, physical disabilities and other symptoms of neurodegeneration. As such, it is a common, often insidious and potentially devastating disorder and an important cause of morbidity and mortality, particularly in the elderly. It accounts for up to a third of all ISs and for about three quarters of all haemorrhagic strokes. It is also the predominant underlying cause of silent cerebral infarcts. In this section, we will describe the ischaemic and haemorrhagic manifestations of small vessel disease, detail the clinical spectrum of CAA and review the association between small vessel disease and cognitive impairment.

1.6.1 Ischaemic manifestations

Manifestations of ischaemia related to small vessel disease are secondary to the development of recent small subcortical infarcts, lacunes and white matter hyperintensities of presumed vascular origin and perivascular spaces.

Recent small subcortical infarcts

Recent small subcortical infarcts attributed to small vessel disease, when symptomatic, will cause a lacunar stroke syndrome. They are thought to account for around 25% of all ISs (Wardlaw et al., 2009) and are associated with increasing age and hypertension. Infarction occurs in the territory of one perforating arteriole with symptoms consistent with a radiological lesion dating from the previous few weeks. They usually present with a sudden onset of motor and/or sensory deficits involving at least two out of the three domains of the ipsilateral face, arm or leg. As a general rule, lacunar syndromes lack findings such as aphasia, agnosia, neglect, apraxia, or hemianopia (so-called ‘cortical’ signs). Monoplegia, stupor, coma, loss of consciousness, and seizures also are typically absent. More than 20 lacunar syndromes have been described. Five have been validated as being highly predictive for the presence of lacunes radiologically (Table 3). The commonest location for each subtype is shown, although symptoms and location do not correlate well and it is not possible to predict the location from the clinical presentation. Of note, larger striato-capsular infarctions represent a separate catergory with distinct cause and should not be attributed to small vessel disease. Similarly, infarcts in the anterior choroidal artery are

aetiologically distinct, identifiable by their location in the caudate nucleus head, and by their shape (mostly comma shaped). Nevertheless, a lacunar stroke syndrome can only be diagnosed in a patient with a classical lacunar syndrome and compatible brain imaging findings based on standardised criteria. Long-term deficits are possible and usually are subtle, mainly in physical and cognitive function.

Table 4. Typical lacunar stroke syndromes verified by autopsy

Clinical Syndrome	Commonest location	Relative prevalence
Pure motor stroke	Internal capsule, pons	45%
Sensori-motor stroke	Thalamus, capsule	35%
Ataxic hemiparesis	Pons, internal capsule	10%
Pure sensory stroke	Lateral thalamus, corona radiata	5%
Dysarthria-clumsy hand	Upper pons, corona radiata	5%

Lacunes and white matter hyperintensities of presumed vascular origin

Lacunes of presumed vascular origin are frequently seen on imaging in elderly patients with no symptoms. Long-term imaging reveals that they are found in 8 to 28% of the general population. Lacunes are associated with an increased risk of stroke, gait impairment, death, and more than double the risk of stroke recurrence and dementia, including AD (Choi et al., 2012; Santos et al., 2009; Snowdon et al., 1997; Vermeer et al., 2007; Norrving, 2008; Arboix and Marti-Vilalta, 2009).

White matter hyperintensities of presumed vascular origin are seen in 7% of the normal population aged between 50 and 69 years, and 17% of the population aged between 70 and 89 years (Barber et al., 1999; Breteler, 2000; Wiszniewska et al., 2000). In other populations, they are seen in 7% of IS patients, 40% of patients with dementia and 60% of patients with vascular dementia. They are more frequent and more severe in vascular dementia and in stroke without dementia than in AD, Lewy Body dementia or controls (Aharon-Peretz et al., 1988; Almkvist et al., 1992; Barber et al., 1999; Charletta et al., 1995). For instance, Erkinjuntti et al. (1987) have found white matter hyperintensities in all their patients with vascular dementia and only in a third of patients with AD. They are associated with covert neurological and cognitive symptoms and physical difficulties such as gait disturbance (de Laat et al., 2011; Windham et al., 2012; Saini et al., 2012; Haley et al., 2009; Inzitari et al., 2009). Similarly to lacunes, white matter hyperintensities are a risk factor for subsequent stroke. In a systematic review of 46 longitudinal studies and of 22 studies included in a meta-analysis, white matter hyperintensities increased the risk of stroke by

3.5-fold (Debette and Markus, 2010). The extent and progression of white matter hyperintensities predicts the risk of a subsequent stroke resulting from arteriolosclerosis (Yamauchi et al., 2002).

Patients with widespread small vessel disease often have severe white matter hyperintensities, multiple lacunes and/or multiple perivascular spaces. The condition was originally referred to as *état lacunaire* or *état criblé* (see section 1.2.1). Clinically, they have recurrent episodes of motor deficit with gait apraxia, impaired balance, and an increased risk of falls. Other symptoms include pseudobulbar palsy and incontinence. Global intellectual deterioration and executive dysfunction are a characteristic feature. The deterioration in gait is associated with the progression and severity of small vessel changes. The mechanisms underlying the gait disturbance remain unclear, although a correlation with frontal lobe atrophy and with WML could imply an interruption of circuits involving the medial frontal lobe that are important in gait control. Physiotherapy to prevent falls has been shown to improve gait and reduce the number of hospitalisations.

1.6.2 Haemorrhagic manifestations

Cerebral microbleeds

CMBs were originally thought to be asymptomatic markers of small vessel disease. Extensive research in the past decade showed that they are associated with other manifestations of small vessel disease and AD (Cordonnier et al., 2007; Vernooij et al., 2008b), and as such, may play an important role in cognitive impairment. Their association with cognitive impairment is increasingly recognised although the mechanisms of this association, i.e by structural damage or dysfunction, are not clearly understood. When confined to the cerebral lobes only, they are a key marker of the presence of CAA; the presence or absence of strictly lobar CMBs has been included in the diagnostic criteria for CAA (Knudsen et al., 2001). CMBs will be extensively studied in the next chapter.

Intracerebral haemorrhage

Major spontaneous haemorrhages are another correlate of brain small vessel diseases and their location may be an indicator of the underlying microangiopathy. Deep haemorrhages in the basal ganglia and deep white matter have been associated with hypertensive microangiopathy (Fisher, 1971a), whereas lobar haemorrhages are more likely to represent the consequences of the rupture of small vessels affected by CAA, especially in the

presence of other lobar haemorrhages or microhaemorrhages (Vernooij et al., 2008b). However, the two types of microangiopathies may coexist, and the presence of a deep or lobar macrohaemorrhage does not exclude the presence of one underlying microangiopathy or the other. Specific clinical manifestations of lobar haemorrhages will be described in section 1.6.3. Examples of patients with deep and lobar haemorrhages are shown in Figure 16. Comprehensive guidelines for the diagnosis and management of ICH have been published previously (Broderick et al., 2007).

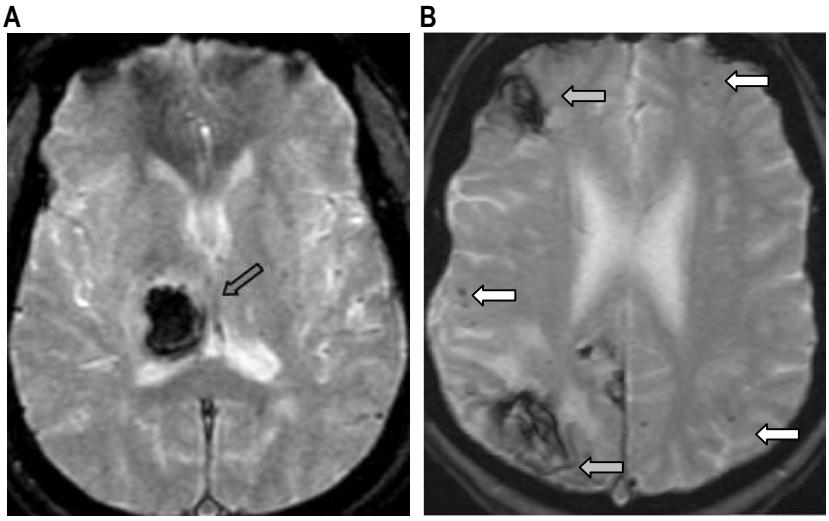


Figure 16. Gradient-recalled T2*-Weighted MRI (GRE T2*-Weighted) in patients with large brain haemorrhages.

A. Deep haematoma in the right thalamus, likely but perhaps not exclusively, related to hypertension. **B.** Two lobar haemorrhages (parietal right, frontal right, grey arrows) are visible in this patient using antiplatelet agents. The image also demonstrates numerous lobar CMBs (white arrows). CAA is likely to be the underlying cause of the CMBs and multiple ICHs in this patient.

1.6.3 Cerebral amyloid angiopathy

CAA is extremely common in normal ageing and is also a frequent, almost invariable component of the disease process in AD. Autopsy studies have demonstrated that the disease is highly frequent in the general elderly population and increases with age, occurring in as many as 50% of individuals in their ninth decade (McCarron MO, Lancet Neurol 2004). The reported prevalence of CAA varies depending on populations and patients studied. Autopsies of patients from a general hospital and from various psychiatric institutions with an average age of 65 years showed a CAA prevalence of 50% (Vonsattel et al., 1991). In a retrospective study of 60 necropsy cases with spontaneous ICH, Ischii found amyloid deposition in 7 cases with a mean age of 77 years; 5 of these were demented (Ishii

et al., 1984). In a review of 15 cases of AD, Mandybur found CAA in 13 cases and noticed a correlation between the presence of amyloid-rich plaques and amyloid angiopathy (Mandybur, 1975). Reported prevalences of CAA in AD vary from 88 to 92%. However, in AD, there is no correlation between the formation of neurofibrillary tangles, the neuritic plaques and the degree of CAA. Vascular amyloid seems to occur independently of plaque formation (Haglund et al., 2006a). Furthermore, different amino acid residue fragments compose neuritic plaques and CAA amyloid (Aβ42 and Aβ40, respectively), suggesting different mechanisms for their processing, accumulation and clearance (Vonsattel et al., 1991).

Outside the context of AD, sporadic CAA is generally recognised in life by the occurrence of lobar cerebral haemorrhages (Sveinbjornsdottir et al., 2008). Since the 1960s, CAA has been suspected as a cause of ICH since not all cases of spontaneous ICH could be explained by hypertension. Neumann was the first to report a fatal cerebral haemorrhage due to amyloid angiopathy (Neumann, 1960). The case was a 46-year-old woman with severe amyloid angiopathy and numerous peculiar senile plaques, who died of a lobar ICH in the left parietal lobe. Fifteen years later, Torack cited two necropsied cases of amyloid angiopathy with massive ICH (Torack, 1975). Jellinger found 8 cases of massive ICH due to CAA among 400 cases of non-traumatic ICH (Jellinger, 1977); seven of them were lobar haemorrhages and one was a basal ganglia haemorrhage, and all had senile plaques and were demented. Okazaki et al. (1979) reported nine cases of massive lobar haemorrhage due to amyloid angiopathy (Okazaki et al., 1979), with all of them associated with senile plaques but only one was clinically demented. All of the cases reported were lobar haematomas. In 1999, Tanaka and colleagues conducted an MRI-histology correlation study of patients with and without intracerebral haematomas. They used T2-weighted MRI and T2*-weighted EPI MRI to detect the ICH and small areas of chronic haemorrhage. Of the 30 patients with ICH, 16.7% were normotensive and normotensive patients also had a high proportion of small chronic haemorrhages (10.3%). Therefore, the authors concluded that other factors besides hypertension were involved in the pathogenesis of cerebral microangiopathy and consequent haemorrhage and they suggested that CAA may be the underlying cause. Nowadays, CAA is considered to be an important cause of lobar haemorrhages and dementia in the elderly (Haglund and Englund, 2002). Based on histopathological examination of 84 cases of spontaneous ICH, Hinton and colleagues estimated that 5 to 10% of all spontaneous ICHs are due to CAA (Hinton et al., 1984). The

haemorrhages are typically lobar and multiple (Gudmundsson et al., 1972; Neumann, 1960).

As demonstrated in the above studies, CAA haemorrhages are peripherally situated in the cortex, subcortex and leptomeninges, reflecting the distribution of the underlying microangiopathy. The disease is most likely to cause lobar (cortico-subcortical) haemorrhages, especially in the occipital and temporal lobes (Rosand et al., 2005), less commonly in the cerebellum (Bruni et al., 1977; Lee and Stemmermann, 1978) and rarely in the deep or brainstem structures. There have been case reports of basal ganglia haemorrhages in normotensive patients with CAA involving the leptomeninges and parenchyma (Ferreiro et al., 1989). In most such instances, other causes such as hypertensive vascular disease must be considered as a more likely cause of haemorrhage. Another characteristic of CAA-related ICH is that the haemorrhages easily rupture into the subarachnoid space (Yamada et al., 1993). SAH associated with CAA is usually secondary to ICH (Yamada et al., 1993) and can be explained by the heavy infiltration of subarachnoid leptomeningeal and cortical blood vessels common in CAA.

Clinically, patients with lobar haemorrhages present with an acute stroke syndrome, with focal neurological deficits that may be associated with headache, nausea, vomiting, seizures and/or an altered level of consciousness, especially in large lobar bleeds. This presentation does not differ from the presentation of lobar haemorrhages secondary to other causes, e.g. arteriovenous malformations, aneurysms and tumours. Patients with secondary SAH due to CAA-related ICH have a higher incidence of headache, vomiting and may have nuchal rigidity. First-ever ICHs in CAA may be clinically mild, but this is counterbalanced by a high risk of recurrent lobar haemorrhages - often in the same lobe as the initial bleed - and by a higher severity of subsequent haemorrhages (Passero et al., 1995). The risk of recurrence in the long term is about 10% per year in elderly cohorts, a higher incidence than in deep ICH (Passero et al., 1995). Negative prognostic factors for a good recovery after lobar ICH are age and a larger haematoma size (Charidimou et al., 2012b).

The relationship of CAA to ICH is not always certain even when CAA is suspected or when its presence has been pathologically demonstrated. Reports have shown a surprising paucity of parenchymal lesions in patients with CAA (Scholz, 1938), whilst in other reports there was a lack of association between the sites of haemorrhage and the location of the

most severe CAA lesions (Vinters and Gilbert, 1983). Multiple factors have been associated with ICH in CAA, including: hypertension (Neumann, 1960; Okazaki et al., 1979), traumatic brain injury (Greene et al., 1990), inflammatory vascular disease (vasculitis or granulomatous angiitis) (Murphy and Sima, 1985; Probst and Ulrich, 1985; Shintaku et al., 1986) and microaneurysms (Bruni et al., 1977; Okazaki et al., 1979; Vonsattel et al., 1991). ICHs occuring in elderly patients may be due to CAA but also to causes other than CAA such as coagulopathies, anticoagulant medications, vascular malformation, or neoplasms. Therefore, in elderly patients without any identifiable vascular disease, it may be difficult to attribute the bleeding to CAA unless specific diagnostic criteria are applied or pathological findings prove the diagnosis.

The most commonly used criteria for CAA diagnosis are the Boston criteria (Table 5). The diagnosis of probable or possible CAA is based on clinico-radiological criteria in the absence of a neuropathological confirmation. The diagnosis of probable CAA requires all of the following: age ≥55 years, the presence of multiple haemorrhagic cerebral lesions confined to cortical or cortical-subcortical (lobar) regions, and the exclusion of secondary causes of ICH. The specificity and sensitivity of the Boston criteria were tested in a population of 39 patients with a clinical and radiological diagnosis of probable CAA against a neuropathological diagnosis (Knudsen et al., 2001). In this study, the specificity of the criteria was 100% (all patients with a probable CAA identified on clinical and imaging grounds had a positive CAA pathology). However, the criteria missed the diagnosis of CAA in 16 out of 29 patients, corresponding to a sensitivity of 44%. Unfortunately, the results of this study are not generalisable to current clinical practice as an important fraction of the patients included did not have a gradient-recalled echo (GRE) T2* MRI, and the severity of the CAA pathology was therefore underestimated by imaging. Recently, the sensitivity of the Boston criteria were improved in a study of Dutch-type hereditary CAA through the greater use of GRE T2* MRI, especially when strictly lobar CMBs were included in the criteria (van Rooden et al., 2009). Superficial siderosis and convexity SAH, both frequent in CAA-related ICH and less common in other forms of ICH, have been shown to increase the sensitivity of the Boston criteria without loss of specificity (Linn et al., 2010). A schematic representation of the clinical manifestations of sporadic CAA is represented in Figure 17. Of note, CAA also occurs in rare genetically transmitted diseases including hereditary cerebral haemorrhage with amyloidosis as described in Icelandic and Dutch families (Esiri and Wilcock, 1986; Gudmundsson et al., 1972; Vinters and Gilbert, 1983; Yamada et al., 1987).

Table 5. Classic and *modified* Boston criteria for the diagnosis of CAA

1. Definite CAA	
Full post-mortem examination demonstrating:	Lobar, cortical or cortical-subcortical haemorrhage Severe CAA with vasculopathy Absence of other diagnostic lesion
2. Probable CAA with supporting pathology	
Clinical data and pathological tissue (evacuated haematoma or cortical biopsy) demonstrating:	Lobar, cortical or cortical-subcortical haemorrhage Some degree of CAA in specimen Absence of other diagnostic lesion
3. Probable CAA	
Clinical data and MRI or CT demonstrating:	Multiple haemorrhages restricted to lobar, cortical or cortical-subcortical regions (cerebellar haemorrhage allowed) *[OR single lobar , cortical or cortical-subcortical haemorrhage and focal[b] or disseminated[c] superficial siderosis] Age ≥ 55 years Absence of other cause of haemorrhage[a]
4. Possible CAA	
Clinical data and MRI or CT demonstrating:	Single lobar, cortical or cortical-subcortical haemorrhage *[OR focal[b] or disseminated[c] superifical siderosis] Age ≥ 55 years Absence of other cause of haemorrhage[a]

[a] Other causes of lobar haemorrhages: head trauma, haemorrhagic transformation of an ischaemic stroke, arteriovenous malformation, haemorrhagic tumour, warfarin therapy with INR >3, vasculitis;
[b] Focal siderosis: siderosis restricted to 3 or fewer sulci;
[c] Disseminated siderosis: siderosis affecting at least 4 sulci

As mentioned in section 1.5, it is also clear that CAA is related to cerebral infarcts (Jellinger, 2002) and white matter pathology (Gravina et al., 1995). Radiologically, amyloid deposition has been associated with CMBs on MRI, and with the presence of cerebral ischaemic changes such as WML and microinfarcts. This will be a specific topic in Chapter 8.

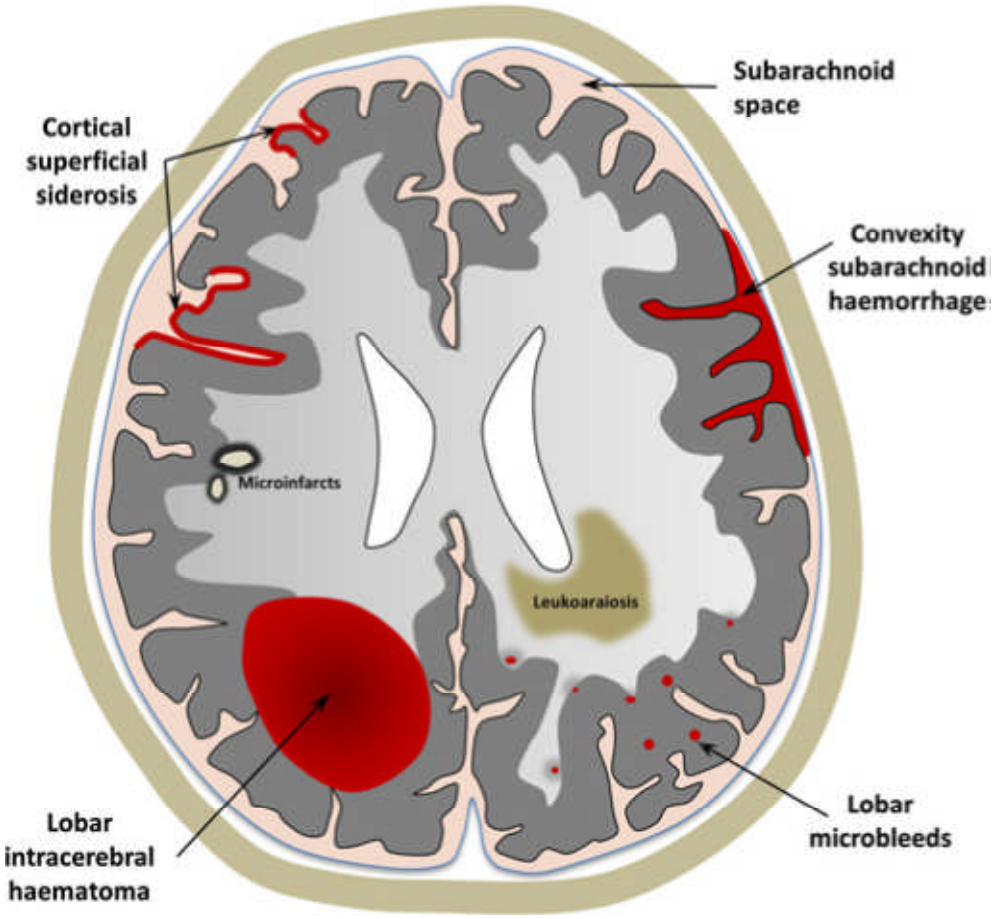


Figure 17. Schematic representation of the spectrum of haemorrhagic and ischaemic manifestations of sporadic CAA visible on MRI.

From Charidimou et al., 2012a

1.6.4 Cognitive impairment

Epidemiology

Cognitive impairment is one of the greatest challenges facing ageing societies. There are currently about 800,000 people with dementia living in the UK and the disease costs the economy £23 billion a year (Department of Health, 2013a). By 2040, the number of people affected is expected to double and the costs are likely to treble. A quarter of hospital beds are occupied by people with dementia. The department of Health's Dementia Challenge was launched in 2012 to achieve better awareness of dementia, increase early diagnosis rates and high quality treatment (Department of Health, 2013b). There are two common causes of dementia: (1) AD (causing up to 60% of cases of dementia) and (2) cerebrovascular disease (causing up to 20% of cases of dementia). The latter is generally

termed vascular cognitive impairment (VCI), a term coined by Hachinski to describe individuals with cognitive difficulties arising from vascular causes (Hachinski, 2007; Rincon and Wright, 2013). Following a stroke, two thirds of patients have some degree of cognitive impairment (Hachinski et al., 2006), which reverses in some patients (Snaphaan and de Leeuw, 2007) but progresses to frank dementia in about a third (Barba et al., 2000). Therefore, early recognition and treatment of VCI are crucial to prevent progression from a 'brain-at-risk' stage, to 'cognitive impairment-no dementia' to the end stage of dementia (Bowler, 2005). Without appropriate preventive measures there is a good chance that one in three people will have a stroke and/or develop dementia during their lifetime (Seshadri et al., 2006). Mounting evidence indicates that vascular and neurodegenerative processes underlying AD and VCI are synergistic, and that the two conditions are probably not separate entities. The frequent co-morbidity of lesions of Alzheimer's disease and cerebro-vascular disease suggests that neurovascular dysfunction could have a major role in the pathogenesis of Alzheimer's disease that by some authors, has even been considered a primary vascular disorder (de la Torre JC, Stroke 2002). The interaction between vascular and degenerative pathologies will be further explained later in this chapter (see pages 70-71 and pages 132-134).

Pathogenic factors and mechanisms

Small vessel disease, which affects the brain diffusely, has been the subject of extensive research as a cause of cognitive impairment, for several reasons: firstly, small vessel disease is very common, and the commonest vascular cause of dementia; secondly, this type of cognitive impairment has relatively homogeneous and clearly defined clinical and radiological features that are potential targets for implementing therapeutic and prevention strategies; and thirdly, with cerebrovascular risk factors as the main cause, early therapeutic intervention might prevent its progression to dementia. Several vascular risk factors (hypertension, hyperlipidaemia, diabetes) and behavioural factors (obesity, sedentarity) have been associated with cerebrovascular disease and dementia in middle-aged and older adults in some studies (O'Donnell et al., 2010). Diabetes and hypertension alone predicted cognitive decline at 6- (Knopman et al., 2001) and 20-year follow-up (Kilander et al., 1998) in otherwise healthy middle-aged individuals. Another study found that diabetes type 2 affects the brain network by disrupting white matter brain connectivity, leading to less efficient transfer of information between brain regions (Reijmer et al., 2013). Several pathogenic mechanisms have been suggested including

dysfunction through pathways mediated by Aβ, BBB alterations (Iadecola et al., 2009), microembolic complications from atrial fibrillation (AF) (Puccio et al., 2009) and potential roles of inflammation (Yamamoto et al., 2005). Active antihypertensive treatment was shown to reduce the risks of dementia (45%) and cognitive decline (34%) associated with recurrent stroke (Tzourio et al., 2003), and the HYVET-COG trial findings support the finding that antihypertensive treatment reduces the risk of dementia (Peters et al., 2008). However, the complex interaction between risk factors severity, WML and load of small vessel disease, VCI and dementia is such that the above associations have been inconsistent between studies. Moreover, there are no unequivocal preventive studies that have established a cause and effect between these. Therefore, it is possible that therapeutic interventions aiming at reducing a person's stroke risk may reduce their risk of developing VCI, but this will need to be confirmed in large prospective studies.

Cerebrovascular lesions related to recent small infarcts, lacunes and white matter hyperintensities cumulatively represent a major contributor to cognitive impairment and physical disability in elderly persons (Thal et al., 2012). Mechanisms have been proposed to understand the cumulative effect of these lesions on cognition, based on the concept of neural nets and brain plasticity (Bowler, 2005; Saczynski et al., 2009). In rich neural networks, disruptions in one circuit following a focal vascular injury may be compensated for by the use of alternative neural connections. Consequently, an initial detriment in efficiency and processing speed will be temporarily observed, and will improve with practice, i.e., learning. As such, the ischaemic damage caused by a few early lesions (particularly in the white matter) may go unnoticed in the absence of clinical deficits; however, as the disease advances, the capacity of the neural net to compensate for the continuing loss of connections decreases. This results in devastating consequences of lesions that occur late in the disease process. Therefore, neural nets offer the potential for recovery but this potential decreases with rising numbers of lesions (Bowler, 2005). The burden of vascular lesions is an important pathogenic factor (Schneider et al., 2003; Vermeer et al., 2003a), as is their location (Benisty et al., 2009; Carrera and Bogousslavsky, 2006; Saczynski et al., 2009). Moreover, this concept would advocate that the reason why frontal executive functions are most prominently affected in VCI (Kramer et al., 2002; Prins et al., 2005) is because of their dependency on complex neural nets (Bowler, 2005; Cummings, 1995). In VCI, the neural nets involved in cognition, memory and behaviour will be affected; these are thalamo-cortical, striato-subfrontal, cortico-subcortical and the limbic systems (Jellinger, 2013a).

Morphological substrates of vascular cognitive impairment

The increasing use of MRI has allowed the identification of markers of cerebral vascular disorders that are linked with VCI; these are lesions related to atherosclerosis of large and medium size arteries, small vessel disease and CAA (Ferrer, 2010). As a marker of small vessel disease, lacunar strokes have been associated with an increased risk of cognitive decline and dementia in longitudinal studies (Miyao et al., 1992; Samuelsson et al., 1996; Yamamoto et al., 2002), and in a meta-analysis (Makin et al., 2013). Because they are part of the spectrum of cerebral small vessel disease, they may carry a higher risk of cognitive impairment than would be expected from their small size, and associated small vessel disease may increase their impact on cognition (Makin et al., 2013). Silent cerebral infarcts incidentally found by CT or MRI have also been linked with cognitive impairment (Vermeer et al., 2007) and poorer long-term outcomes (Pantoni, 2003), suggesting that the term 'silent' may be misleading.

Likewise, it is well established that WML are associated with cognitive decline (Jokinen et al., 2005) and dementia (Pantoni, 2010; Jokinen et al., 2009; Prins et al., 2004). In the LADIS study, severe white matter hyperintensities independently and strongly predicted rapid global cognitive decline of older adults with non-disabling complaints three years later (Inzitari et al., 2009). A correlation was found between progression of white matter lesion load and a decline in cognitive performance (Schmidt R, Stroke 2007), particularly affecting information processing speed (van Dijk et al., 2008). These observations were recently confirmed in a large pathological population-based study of 456 brains, including brains with pathological features of AD, cerebral atrophy and vascular disease; pathology was correlated to dementia status based on information obtained prior to death (Savva et al., 2009). In this study, the presence of small vessel disease was associated with a more than two-times increased risk of dementia at the age of 75 years. A threshold of vascular damage, necessary for the expression of subtle cognitive deficits, was demonstrated in elderly subjects who have otherwise a few cerebrovascular lesions without cognitive deficits (Jellinger, 2013a; Pantoni, 2010).

Finally, there is increasing evidence that CAA is an important contributor to cognitive impairment. CAA is clinically and pathologically associated with AD; the presence of the amyloid protein is a pathological hallmark of the disease in which it is almost invariably seen (Jellinger and Mitter-Ferstl, 2003). Population-based clinico-pathological studies have shown that the presence of CAA is higher in demented compared with non-demented

patients (Keage et al., 2009). Severe CAA was much more frequent in neuropsychiatric patients (26.3%) than in general hospital patients (3.3%) in a comparative histopathological study of a hospital-based population (Vonsattel et al., 1991), thereby suggesting that CAA plays an important role in cognitive performance. CMBs, a radiological marker for small vessel pathologies including CAA detected on gradient-echo T2* MRI sequences (Fazekas et al., 1999) may play a key role in cognitive impairment. They are associated with executive dysfunction in stroke patients (Werring et al., 2004) and with dementia severity in patients with subcortical cognitive impairment (Seo et al., 2007). We have conducted 2 studies to assess their cognitive impact in stroke patients which are detailed in Chapter 7. The close relationship between CAA, coexisting AD and other age-related pathologies (e.g. hypertensive arteriopathy) poses a challenge to the study of the independent effects of CAA and CMBs on cognition. This will be further discussed in Chapter 7.

Clinical aspects

VCI is difficult to diagnose, due to moderate sensitivity and specificity of the multiple published diagnostic criteria, and the lack of morphological criteria (Jellinger, 2013a). This contrasts with the recently refined diagnostic criteria for AD (Jellinger, 2013b). Clinically, vascular brain injury causes dysfunction in regions important for memory, cognition and behaviour. The cognitive deficit in VCI differs from AD with greater executive and attentional dysfunction (deficits in information processing speed and executive function) and relatively intact episodic memory. White matter pathology in frontal-basal ganglia circuits has frequently been invoked as an important cause of executive dysfunction. In healthy subjects, large white matter hyperintensities were found to be associated with impaired basic attention, visuo-constructive abilities, mental speed and executive control (Almkvist et al., 1992; Au et al., 2006; Boone et al., 1992; Breteler, 2000). White matter volume was also associated with urinary incontinence, the grasp reflex, and abnormal motor behaviours in patients with AD (Hirono et al., 2000).

Interaction between vascular and degenerative pathologies

There is increasing evidence of an association between cerebrovascular disease and AD. Dementia after stroke is very common (Henon et al., 2006; Leys et al., 2005), and related to presence of pre-stroke AD pathology (Henon et al., 1998; Linden et al., 2004). Cerebrovascular lesions often coexist with Alzheimer-type lesions: 25-80% of elderly demented show mixed pathologies (Jellinger, 2013a), and they often have a large variety of

different vascular lesions (Schneider et al., 2007). CAA, a vascular disorder of the small vessels, is associated with cognitive impairment and is present in up to 100% in AD brains (Greenberg et al., 2004b). It is possible that mild Alzheimer dementia pathology and small vessel disease interact synergistically in promoting dementia (de la Torre, 2002; Barba et al., 2000), and AD has even been considered a primary vascular disorder by some authors (de la Torre, 2002). Common etiologic and reciprocally synergetic pathophysiological mechanisms may promote both pathologies (Park et al., 2013; Yarchoan et al., 2012). Several studies have observed that in patients with cerebrovascular disease and AD, for the same severity of dementia, there were significantly fewer neurofibrillary tangles (one of the major histopathological hallmarks of AD) in the brain, compared with patients with AD only (Etiene et al., 1998; Zekry et al., 2002). This finding suggests that vascular lesions and Alzheimer's lesions have synergetic effects in the exacerbation of the clinical manifestations of AD. None of the cases showed vascular disease without the presence of Alzheimer's lesions, suggesting that either AD or vascular lesions alone may be insufficient to induce dementia. Likewise, cerebrovascular disease may play an important role in determining the severity of AD symptoms, also in keeping with a possible synergistic interaction between both pathologies (Snowdon et al., 1997). In elderly patients with subclinical or mild AD, critically located vascular lesions or cortical watershed infarcts may shorten the period of preclinical disease (Esiri et al., 1999; Pasquier and Leys, 1997) and worsen cognitive impairment in established disease (Esiri et al., 1997; Miklossy, 2003). In late stages of AD, vascular lesions have less impact on the progression of the cognitive decline (Jellinger, 2007). Therefore, VCI is likely to have a multifactorial etiology; vascular lesions, Alzheimer's pathology and aging have a cumulative effect on the risk threshold of damage required to induce dementia. The mechanisms by which these many factors operate, and whether they are independent or synergistic, remain incompletely understood.

Chapter 2 - Introduction to Cerebral Microbleeds

The objectives of this chapter are to provide a historical overview of CMBs, to understand the principles of their detection by MRI, to emphasize the importance of standardised reporting and to describe their epidemiology and risk factors in different populations.

2.1 From microaneurysms and haemosiderin deposits to cerebral microbleeds

CMBs are MRI-defined lesions representing previous small areas of blood-degradation product deposition in the brain, usually considered to result from rupture of diseased small vessels. They have generated intense interest as radiological markers for small vessel diseases prone to bleeding, with the hope that they might contribute to our understanding of the mechanisms of small vessel diseases, to non-invasively diagnosing small vessel diseases in life, and to serving as a prognostic marker of larger ICHs. Understanding the causes of spontaneous ('primary') ICH (or ICH without a clear cause) is of critical importance because this type of stroke has devastating effects in terms of mortality and morbidity. The underlying pathological processes of spontaneous ICH have been an important topic of investigation for centuries. It is clear from many studies that spontaneous ICH relates to characteristic pathological changes to the small vessels; these are hypertensive arteriopathy and CAA (see Chapter 1). In addition to these, many old histopathological studies have described small aneurysmal lesions or perivascular haemorrhages in association with larger haemorrhages, which may be relevant in understanding the origin of radiologically-defined CMBs. The term 'microbleeds' was first coined in 1996 to describe small areas of bleeding in the brain (Offenbacher et al., 1996), and many different terms have been used in subsequent years. We have chosen to use the most commonly used term, *cerebral microbleeds*, in this thesis. After a brief historical introduction, we will describe the histopathological lesions that underlie their characteristic radiological appearance. We will then discuss the methods to detect, define and map CMBs and their radiological characteristics in a second section, and will end this chapter with a discussion on their prevalence among various populations and risk factors.

2.1.1 Charcot-Bouchard microaneurysms

In '*Observationes anatomicae ex cadaveribus quos sustulit apoplexia'* published in 1658, the Swiss pathologist Johann Jacob Wepfer (1620 – 1695), was the first to hypothesise that the effects of a stroke were caused by bleeding in the brain (Compston, 2005). From post-mortem studies, he found fragile, thickened vessels with degenerative changes secondary to hypertension in relation to large cerebral haemorrhages, but was unable to identify a point of rupture. The question of the primary site of bleeding in cases with no apparent

ruptured major artery or vein had never been demonstrated until the research by Jean-Martin Charcot and Charles-Joseph Bouchard opened new perspectives (Figure 18).

Figure 18. Left panel: Jean-Martin Charcot (1825-1893). Right panel: Charles-Joseph Bouchard (1837-1915).

Jean-Martin Charcot was a French neurologist and professor of anatomical pathology at the famous Salpêtrière Hospital in Paris for more than 30 years. He is known as 'the founder of modern neurology' and his name is associated with several medical eponyms including Charcot-Marie-Tooth disease and amyotrophic lateral sclerosis (motor neurone disease). One of his interests was research on the functions of different parts of the brain and the role of arteries in cerebral haemorrhage. Charles-Joseph Bouchard was a student of Jean-Martin Charcot, and with Charcot he described the miliary aneurysms now known as Charcot-Bouchard aneurysms. Bouchard wrote about these aneurysms in his doctorate thesis '*Étude sur quelques points de la pathogénie des hémorrhagies cérébrales.*'

In 1868, Charcot and Bouchard carefully examined the brains of 60 patients, mostly elderly, who had died from ICH, and found what they interpreted to be miliary aneurysms on water macerated vessels measuring 250-400 μm in diameter (Charcot and Bouchard, 1868). These microaneurysms, which still bear their names, were regarded as the potential cause of cerebral haemorrhage as they were present in nearly 90% of patients who had suffered a massive ICH. They were 200-2000 μm in diameter, saccular or fusiform dilatations attached to vascular filaments in the walls of the ICHs. They concluded that such changes were the result of 'periarteritis' (rather than conventional arteriosclerosis) and suggested that miliary aneurysms might be responsible for hypertension-induced ICH.

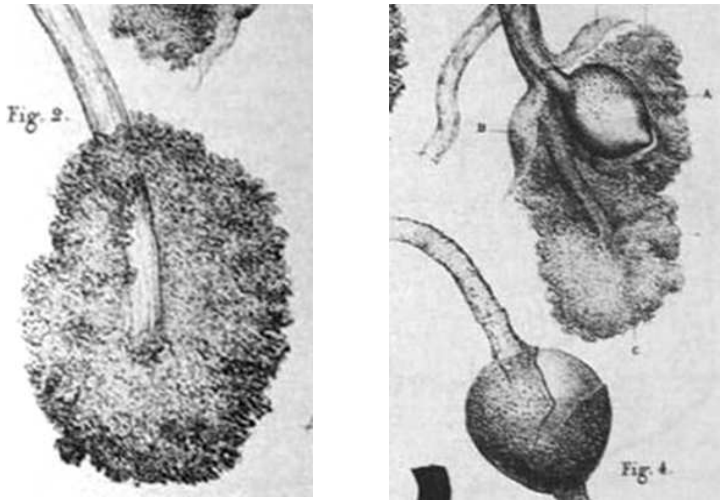


Figure 19. Original illustration of 'miliary parenchymal aneurysms' by Charcot and Bouchard (1868).

Left panel shows an aneurysm with adjacent leakage of blood; right panel shows an aneurysm without leakage, and a collection of blood without aneurysm.

However, despite their revolutionary findings, many scientists in subsequent years doubted the authenticity of Charcot and Bouchard's findings because the original study was limited by several methodological issues: first, the details of the microscopic description were lacking; second, rinsing the haemorrhage in water then microscopically examining teased filaments might have precluded the reconstruction of the vascular anatomy; and finally, the exact composition of the walls of the vessels and aneurysms and the nature of the luminal contents were not discernible because of the low magnification used. These issues generated controversy about the true existence of miliary aneurysms. There had never been any clinico-pathological confirmation that miliary aneurysm rupture caused cerebral haemorrhages. Nevertheless, by the end of the nineteenth century, none of the possible causes of cerebral haemorrhage other than arterial rupture (perivascular autolysis of nervous tissues, haemorrhage into areas of infarction due to adrenalin, confluence of petechial extravasations, bleeding due to rupture of cerebral veins) were widely accepted, and Charcot and Bouchard's findings forged the generally accepted idea that pathological changes (degenerative or inflammatory) in small calibre arterioles associated with hypertension cause weakening of the wall and microaneurysm formation, which subsequently ruptures to cause ICH. Their work generated exponential research into the mechanisms of hypertension-induced ICH. A summary of selected studies on microaneurysms in association with hypertension and ICH is shown in Table 5.

Many subsequent studies suggested alternative explanations for microaneurysms, including dissection of blood into a diseased segment of arterial wall, false aneurysms (also referred to as 'bleeding globes') or even misinterpretation of complex arteriolar loops or coils as aneurysms. In 1882, Charlewood Turner did not find microaneurysms in association with ICH. He suggested that the dilatations found by Charcot and Bouchard resulted from the accumulation of blood between the media and adventitia, and that microaneurysms might be dissecting. This theory was later followed by Eppinger (1887) and Professor A.G. Ellis (1909). In 1909, Professor A.G. Ellis found microscopic aneurysms in 20 cases of hypertension-related ICH and attributed them to dissection of the vascular wall. He sustained that miliary aneurysms arise from the rupture of the damaged intima allowing intramural dissection of blood and subsequent medial degeneration. His findings suggested for the first time a link between progressive degenerative changes of the vessel wall following an initial intimal lesion as the cause of spontaneous hypertension-related ICH. In some cases, perivascular haematomas in continuity with the intramural contents gave the appearance of red, grape-like swellings which Ellis thought could have been mistaken for true microaneurysms. Ellis' work was interpreted as indicating that miliary aneurysms are in fact periadventitial haematomas or pseudoaneurysms formed by blood clots adherent to vessels. Several authors found similar lesions, resembling aneurysms but in reality corresponding to masses of red blood cells adjacent to vessels in the walls of haemorrhages. They were referred to as 'bleeding globes', a term introduced by Matsuoka (Matsuoka, 1939), and were thought to result from small extravasations from arteries torn apart by the main haemorrhage (Beitzke, 1936) or relate to functional rather than mechanical circulatory disturbance (Wolff, 1937). In 1971, Miller Fisher (1971a) also clearly demonstrated 'bleeding globes' at each site of arterial bleeding in microscopic sections of ICHs, structures without outer walls and formed by fibrin envelopes, thereby artefactually creating the appearance of true aneurysmal dilatations. He was not convinced there were any aneurysms in the microscopic sections, and supported the idea that aneurysms are nothing more than perivascular collections of blood clots, or tiny extravasations of blood adherent to small arteries, or perhaps external dissections into the adventitia.

In 1930, F.H.K. Green made similar observations to Ellis. He took multiple blocks of brain tissue from 10 hypertensive subjects and examined serial sections under the microscope (Green, 1930). The brains had been hardened in formol saline for a few weeks prior to cutting blocks into thick sections to preserve the course of visible arteries. Using this technique, he found three microaneurysms consisting of a stretched and ruptured intima

and media with distension of the adventitia and effusion of red blood cells in the perivascular space. As such, he not only confirmed the existence of microaneurysms, but was also the first to speculate that the cause of microaneurysm formation was arteriosclerosis rather than 'peri-arteritis'. However, one can question the authenticity of these lesions given the few lesions found and the very limited method of investigation.

Favouring Charcot and Bouchard's views on microaneurysms, Dr Ralph Ross Russell, who had been working with Sir George Pickering in Oxford on the cause and effects of hypertension, set out to confirm the presence of miliary aneurysms as the primary event in ICH using a more comprehensive and systematic technique, the arteriolar X-ray micro-angiography (Ross Russell, 1963). This consisted of a post-mortem angiography using immediate perfusion of the carotid arteries with barium sulphate in gelatine followed by cooling and formalin fixation. He found a strong correlation between hypertension and the presence of intracerebral aneurysms, with 14 out of 15 hypertensive brains presenting aneurysms compared with 10 out of 35 normotensive brains (Figure 20).

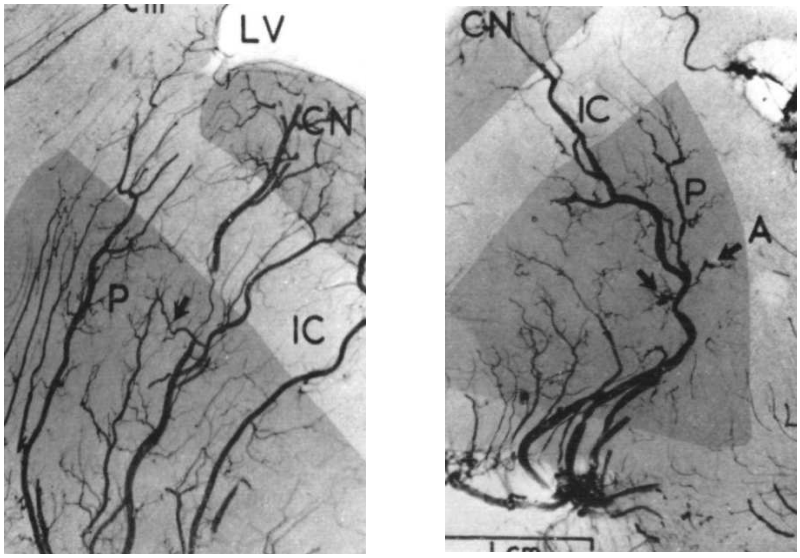


Figure 20. Coronal radiographs of the lenticulo-striate arteries from a young normotensive subject and from a hypertensive subject.

In the normotensive subject (left panel), the branches distribute evenly and regularly without irregularity. Attenuation of small vessels and saccular dilatations (arrowed) are visible in the hypertensive subject (right panel). IC, internal capsule; P, putamen.
From Ross Russell, 1963

Up to 20 aneurysms were present in any one individual, and multiple aneurysms were seen in hypertensive patients only. He found saccular dilatations along small lenticulo-striate perforating arteries of 100-300 µm in diameter, especially at bifurcation points of arterial branches, and in well-defined sites of the brain (basal ganglia, centrum semi-ovale and cortical grey matter) (Figure 21). Microaneurysms consisted of an internal layer derived from the intima and an outer collagenous layer continuous with the adventitia of the parent vessel. He interpreted this degeneration of the muscular and elastic component of cerebral arteries as the result of the combined effects of age and hypertension. Interestingly, there were often some red blood cells outside the aneurysms, with many iron-containing macrophages and haemosiderin staining; an observation of definite relevance for the understanding of the origins of CMBs. The aneurysms were present in the absence of recent or past haemorrhage in the vicinity, strongly supporting the fact that they were the cause and not the result of ICH.

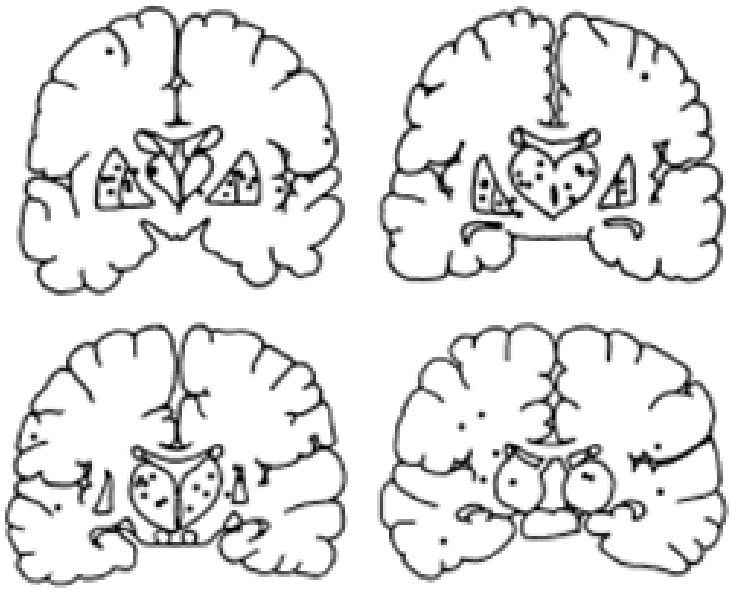


Figure 21. Distribution of microaneurysms in 14 hypertensive patients in multiple coronal sections. The brainstem and cerebellum were not included

From Ross Russell, 1963.

A few years later, Cole and Yates (1967) studied 200 brains, including 100 hypertensive and 100 normotensive brains, from age and sex-matched subjects in a post-mortem study using an injection technique similar to that used by Ross Russell. Forty-six percent of the hypertensive brains showed aneurysms, compared with 7% of the normotensive brains. Brains from 13 hypertensive subjects demonstrated small areas of bleeding outside the vessel wall. The microaneurysms were surrounded by fresh blood and haemosiderin pigment, once again providing a pathological lesion relevant to the study of CMBs. Although the age of their population was slightly younger than that of Ross Russell, they proposed that miliary aneurysms were the causal link between hypertension and minor haemorrhage, but were unlikely to cause the massive variety of ICH observed. Indeed, only a minority of aneurysms occur on large vessels, and hypertensive haemorrhage can occur in the absence of aneurysms. They further suggested that subclinical rupture of small arteries and reabsorption of haemorrhages can form small cystic cavities where miliary aneurysms later develop. However, the injection technique using barium sulphate had serious drawbacks which led subsequent brain scientists to doubt Cole and Yates and Ross Russell's findings: first, small aneurysms are difficult to recognise in angiograms even when magnified because this technique only demonstrates aneurysms that are above a certain size and have relatively empty lumens; second, the exact aneurysm size is difficult to measure due to large lesions sometimes being filled with thrombus; and third, it may

overestimate the presence of aneurysms because the injection creates artificial bulges and leaks due to injection pressures.

In subsequent years, the debate continued on whether arterial rupture resulted from miliary aneurysms or from weakening of the arterial wall by arteriosclerosis. Further studies looking specifically at the ruptured portion of the arterial wall were necessary. In 1972, Miller Fisher found miliary aneurysms in the necropsy brain from hypertensive patients with a history of stroke including macroscopic ICH (Fisher, 1972). Blood coated the outside of some aneurysms, and haemosiderin-laden macrophages were scattered in the surrounding tissue suggesting chronic haemorrhage. All aneurysms were associated with hypertension, and affected small arteries showing lipohyalinotic disorganisation. He hypothesised that the microaneurysms could not individually cause macroscopic ICH due to the small size of the parent artery, and that lipohyalinosis might be the common pathogenic mechanism to miliary aneurysms and macroscopic ICH. Many of the lesions had obliterated vascular lumens, and vascular weakening and rupture had to occur before the formation of the well-developed lipohyalinotic aneurysm.

In 1983, Takebayashi and Kaneko provided the first investigation of surgical specimens as a way to identify the cause of hypertension-induced ICH (Takebayashi and Kaneko, 1983). They performed ultrastructural observations of 20 ruptured lenticulo-striate arteries obtained from surgical material collected during emergency surgery for hypertensive ICH and of 11 freshly removed brains of hypertensive patients who had died from ICH. They found two ruptured miliary aneurysms and five non-ruptured aneurysms (Figure 22). Arterial rupture was identified in 11 lenticulo-striate arteries measuring less than 300 µm, and in 10 autopsy cases each showing 2 to 11 ruptured sites on arteries of the large variety (500-700 µm). Ruptured arteries characteristically showed hypertension-related degeneration of the medial smooth muscle cells and dilatation of the vascular lumen indicating a weakening of the vascular wall due to a loss of elasticity. Their theory was that aneurysms resulted from induced dissection at bifurcations, sites highly susceptible to tensile forces. Whether rupture was complete or incomplete and resulted in bleeding, depended on intravascular pressure, stress forces and the size of the arteries. In addition, there was a positive correlation between the size of the haematoma and the size and number of ruptured arteries, suggesting that miliary aneurysms are not likely to cause rupture. At the sites of rupture, abrupt breakage of the arterial wall was more common than microaneurysms. Therefore, they concurred that a large ICH is due to the rupture of

large arteries, sometimes multiple ruptured arteries. The rupture of a single vessel gives a variety of tensions to the adjacent arteries inducing rupture of unruptured arteries around the large haematoma in an avalanche fashion. These findings were in agreement with Miller Fisher's emphasis on lipohyalinosis rather than microaneurysms as a common cause of vessel rupture.

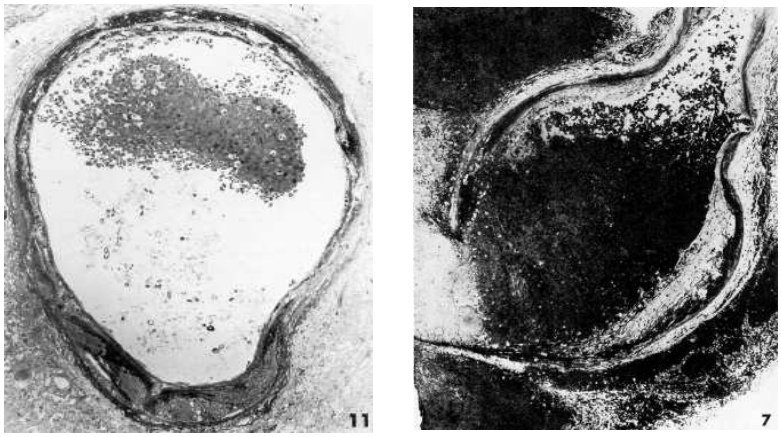


Figure 22. Non-ruptured military aneurysm and ruptured military aneurysm.

The unruptured aneurysm (left panel) measures 500 μm in diameter. The wall of the aneurysm consists of dense layers of fibrin and plasma protein mixed with a few haematogenous cells. The ruptured aneurysm (right panel) measures 1000 μm in diameter. Its wall contains dense band-like deposition of fibrins. The connecting artery at the right upper corner measures 180 μm in diameter and shows atherosclerotic changes
From Takebayashi and Kaneko, 1983

In 1989, Wakai and Nagai provided a second investigation in which surgical specimens were used to identify the cause of hypertension-induced ICH, which re-emphasised the role of microaneurysms as a source of bleeding. They proceeded with a thorough investigation of the haematomas and their walls under surgical microscopy in specimens from 14 patients with spontaneous lobar ICH or cerebellar ICH. In the periphery of the haematoma, miliary aneurysms were present in five cases and possible miliary aneurysms in two cases; possible microaneurysms were identified in tissues in which the parent artery was not verified histologically. There was contiguity between the masses of red blood cells and the surrounding haematomas in all cases, and an arterial connection between the solid tissue and the blood clot was found in all but one, suggesting that a vascular abnormality, likely a microaneurysm, was the cause of bleeding.

Favouring the idea that ICH results from other sources than microaneurysms, Mituzani and coworkers provided beautiful illustrations of arterial dissections of lenticulo-striate arteries from surgical specimens in 12 hypertensive patients with ICH (Mizutani et al., 2000). They identified 6 arterial dissections of 15 arteries measuring 100-700 µm in diameter, suggesting that intracerebral arterial dissections may be a much more common cause of hypertension-related ICH than previously thought.

A study using a new histochemical technique raised significant doubts about the authenticity of miliary aneurysms in the early nineties. Challa and co-workers (Challa et al., 1992) reassessed the existence of Charcot and Bouchard microaneurysms using Gomori's alkaline phosphatase to stain the endothelium lining of microvessels of post-mortem brain specimens from 35 hypertensive and 20 age-matched normotensive patients. The sections were examined using light microscopy and high resolution microradiography. This contrast-free technique provided three dimensional perspectives with the enhanced ability to trace vessels, and eliminated the artefacts of injection and maceration methods. The team also sampled 2800 brains via routine paraffin sections. Charcot-Bouchard microaneurysms were conspicuously absent in both groups which included four cases of hypertensive ICH. The routine paraffin sections demonstrated only five examples of parenchymal aneurysms, all in elderly patients with advanced arteriosclerotic changes and none were associated with ICH. In contrast, the technique demonstrated tortuous vascular profiles and identified complex coils and twists in arterioles, especially in old age (Figure 23).

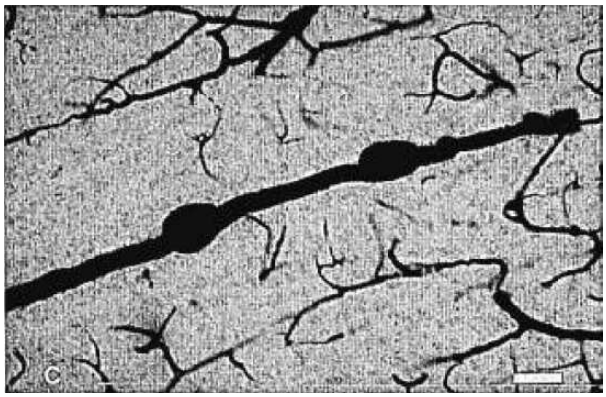


Figure 23. Photomicrograph showing complex microaneurysm-like vascular coils and twists revealed using alkaline phosphatase staining.
From Challa et al., 1992

These complex structures can mimic microaneurysms when assessed using old techniques (paraffin and injection techniques) and lead to misinterpretation. Their impression was that Charcot and Bouchard microaneurysms have little relationship to ICH for several reasons: first, parenchymal aneurysms show chronic changes of fibrosis and thrombosis which cannot be linked with acute events such as ICH; second, most sections when taken from the edges of large haemorrhages do not usually show aneurysms; third, fibrinoid necrosis of arterioles in the vicinity of ICH is a reaction to extravasated blood; and finally, the drawbacks of previous methodological techniques of identification, namely overestimating (injection technique) or underestimating (paraffin studies of thin slices) the presence of microaneurysms.

However, the suggestion of such misinterpretation does not explain the preferential location of microaneurysms in the basal ganglia (Ross Russell, 1963). Furthermore, autoradiographic studies used systematic analysis of the full thickness of the sections combined with histopathological analysis, making misinterpretation unlikely. It is also possible that aneurysm frequency has been underestimated in Challa and coworkers' study (1992) because alkaline phosphatase is absent from severely damaged aneurysmal arteriolar walls. Nevertheless, it appears from their study that microaneurysms are likely much less common that previously thought, and that rupture of non-aneurysmal segments of arterial walls damaged by hypertension, is the most likely mechanism of hypertension-related ICH.

Only scarce reports have provided direct histological evidence for ruptured microaneurysms as a clear source of bleeding, which may explain the continued controversy on the existence of miliary aneurysms and their role in hypertensive haemorrhage. In 2003, Miller Fisher reported the case of a thalamic haemorrhage which had been sectioned serially in its entirety revealing a single site of bleeding. The ICH had occurred in a hypertensive patient treated with pitressin for gastrointestinal bleeding-related hypotension. Microscopical examination revealed that the ICH arose from an elongated aneurysmal segment of a small artery measuring 180 µm in diameter. By following the aneurysmal outline in the serial sections, a 1 mm wide breech was identified through the fibrillar material of the retaining wall which allowed continuity between the lumen and the extravasated blood of the ICH. In the same year, Rosenblum provided an example of a ruptured aneurysm within a pontine haemorrhage in an 83 year old male patient, but in a very unusual context: the aneurysm had undergone dissection at its point

of origin at a site of fibrinoid necrosis, and the outer wall of the dissection had ruptured, causing parenchymal haemorrhage (Rosenblum, 2003). This was the first evidence of dissection within an aneurysmal wall. The authors explained this phenomenon by the fibrinoid necrosis that had weakened the wall of the parent arteriole leading to the aneurysmal dilatation and dissection, with the haemorrhage resulting from the reduced resistance to rupture of the thin outer wall of the dissection.

In conclusion, to date, many studies have not been able to consistently find true microaneurysms at the site of macroscopic ICH. A combination of significant methological issues and normal physiological events (e.g. destruction of microaneurysms during the acute ICH) hindered the comprehensive assessment of microaneurysmal lesions as a cause of bleeding. Rare reports have demonstrated the presence of microaneurysms at the core of the arterial rupture. Nevertheless, many histopathological studies have demonstrated that important structural changes, with or without microaneurysms, occur within and in the vicinity of small perforating arteries of the brain exposed to chronic hypertension. These changes are likely to play a key role in the pathogenesis of large symptomatic ICH. Among these, blood degradation products possibly resulting from chronic extravasation through damaged vessel walls can be seen in the perivascular space, and might be the underlying substrate of radiologically-defined CMBs. Therefore, based on the best available evidence to date, there is no doubt that Charcot and Bouchard microaneurysms occur, and a significant role of microaneurysms in ICH cannot be rejected. However, many fundamental questions remain regarding their cause, nature, prevalence and role in the pathophysiology of ICH. To cite Miller-Fisher, haemorrhage entails not only the initial rupture of a blood vessel, with or without microaneurysmal rupture, but also the secondary mechanical shearing of surrounding blood vessels allowing the ICH to reach its full size (Fisher, 1971a).

Table 6. Summary of selected studies on microaneurysms (Charcot-Bouchard aneurysms) in association with hypertension and ICH

Adapted from Werring, 2011

Study	Year	Technique	No. of brains	Prevalence of microaneurysms		
				Controls	Hypertension	Intracerebral haemorrhage
Green	1930	Microscopic examination of serial frozen & paraffin sections of blocks of brain tissue	10	-	1/7 (14%)	1/3 (33%)
Ross Russell	1963	Arteriolar X-ray micro-angiography with barium-gelatin injection	54	10/38 (26%)	14/16 (88%)	5/5 (100%)
Cole and Yates	1967	Arteriolar X-ray micro-angiography with barium-gelatin injection	200	7/100 (7%)	46/100 (46%)	18/21 (86%)
Fisher	1972	Microscopic examination of formalin-fixed blocks of brain tissue	20	-	13/20 (65%)	NA
Takabayashi and Kaneko	1983	Electron microscopy of thin and semi-thin consecutive sections of Epon 812 embedded arteries	61	-	-	7/61 (11%)
Wakai and Nagai	1989	Meticulous microsurgical technique followed by serial sectioning and microscopic examination	14	-	-	5/14 (36%)
Challa et al.	1992	Bells' modification of the alkaline phosphatase technique	55	0/20	0/31	0/4
Mizutani et al.	2000	Microscopic examination of surgical specimens	12	-	-	0/12
Overall prevalence				**17/158 (11%)**	**74/174 (43%)**	**36/120 (30%)**

2.1.2 The first MRI reports of 'microbleeds'

Although macroscopic ICH can be seen on non-contrast CT scans, the small aneurysmal lesions and perivascular haemorrhages discussed above cannot be visualised on conventional neuroimaging methods; even the gold standard of catheter angiography is not sensitive enough to detect vascular lesions of this size. It has, therefore, been impossible to visualise such lesions *in vivo* (Singh Alg and Werring, 2011). *In vivo* investigation of these lesions has not been possible until the advent of MRI. Scharf et al. (1994) reported the presence of small, intracerebral dots of marked signal loss using T2-weighted Fast Spin Echo (FSE) MRI in patients with hypertensive cerebrovascular disease and ICH. These seemed to be associated with ICH, lacunar infarcts and ischaemic white matter disease, and they called them 'haemorrhagic lacunes'. It is likely that these lesions were more substantial haemorrhages (possibly secondary haemorrhagic transformation of lacunar infarcts), since they used a T2 sequence at low magnetic field strength (1.0 tesla). Since the mid-1990s, very small areas of bleeding have become detectable using a sequence highly sensitive to haemosiderin, gradient-echo T2*-weighted MRI. These lesions appeared as small, rounded dots of low signal intensity in patients with ischaemic and haemorrhagic stroke, and in hypertensive and healthy elderly subjects (Fazekas et al., 1999; Roob and Fazekas, 2000). In 1996, Greenberg et al. reported 'petechial haemorrhages' in 9 out of 15 patients with lobar haemorrhages consistent with CAA (Greenberg et al., 1996). In the same year, Offenbacher and colleagues described similar intracerebral foci of MR signal loss in 39 of 120 patients with spontaneous ICH (Offenbacher et al., 1996). The majority consisted of homogeneous, rounded lesions with diameters of 2 to 5 mm, and they termed these 'microbleeds'. Figure 24 is taken from the same paper, and demonstrates the difference in sensitivity for the detection of small and large haemorrhagic lesions between non-contrast CT, T2-weighted fast spin echo (FSE) MRI and gradient-echo T2*-weighted MRI (Figure 24). Both the studies by Greenberg and Offenbacher demonstrated a strong association between ICH and CMBs on gradient-echo T2*-weighted MRI, suggesting that CMBs may provide key information into the pathogenesis of ICH.

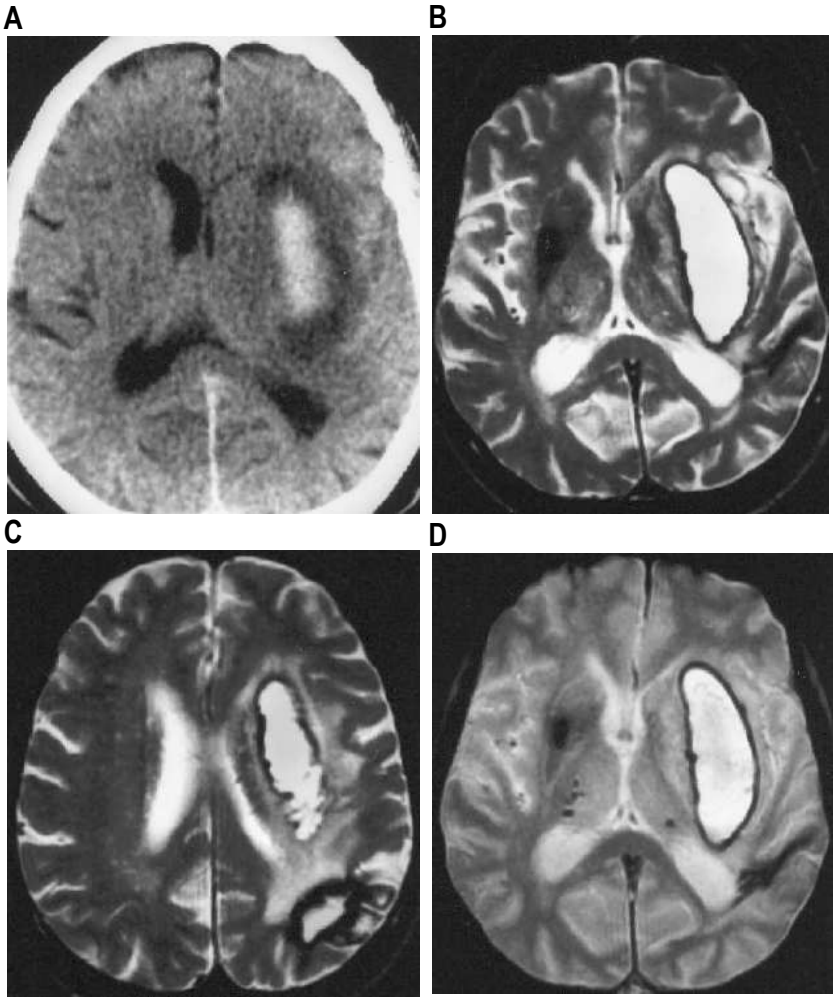


Figure 24. CT and MR imaging of a 74 year old patient with a putaminal spontaneous ICH showing the discrepancies between the different imaging modalities.

A. CT scan shows an acute haematoma without any other parenchymal abnormalities. **B.** and **C.** T2-weighted spin-echo MR images reveal another clinically silent old haematoma in the left parieto-occipital region not detected by CT. **D.** T2*-weighted GRE MRI permits better visibility and shows many additional CMBs.
From Offenbacher et al., 1996.

In the same year, Chan reported multifocal hypointense cerebral lesions on gradient-echo T2*-weighted MRI of patients with chronic hypertension. All seven patients in whom MRI had shown small chronic haemorrhages had also been chronically hypertensive (Chan et al., 1996). These small haemorrhages were located in the basal ganglia, thalamus, corona radiata and subcortical white matter, brainstem, and cerebellum (although cortical foci were not counted formally). Although other factors may have contributed to the apparent lesions, this study demonstrated an association between multifocal hypointense lesions found on MRI and a history of chronic hypertension. A correlation was established between

the location of CMBs, and the areas involved in lacunar infarction, leukoaraiosis and cerebral haemorrhage (Figure 25).

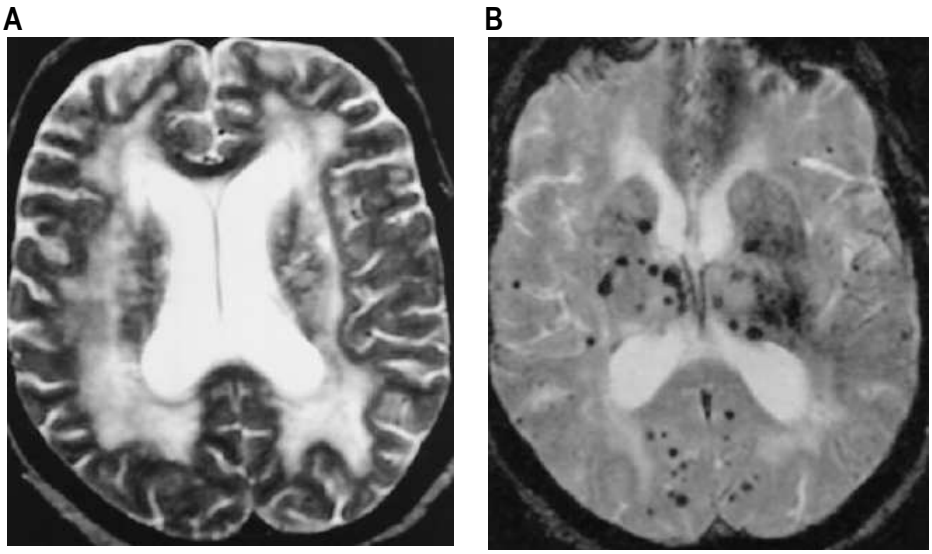


Figure 25. MR imaging of a patient with chronic hypertension and worsening dementia showing the overlap between white matter changes and CMB distributions.

A. T2-weighted fast spin-echo MR image shows extensive white matter hyperintensity suggestive of leukoaraiosis. No hypointense lesion is identified. **B.** T2*-weighted GRE MR image shows multiple hypointense lesions (CMBs) with a predominantly deep distribution. The distribution of white matter changes and CMBs clearly overlaps. From Chan, 1996

2.1.3 Histopathological correlates of cerebral microbleeds

In 1999, the histopathological correlates of CMBs began to be reported. Histological-MRI correlations demonstrated that the hypointensities detected on GRE T2* images reported as CMBs correspond to focal deposits of paramagnetic blood break-down products, especially haemosiderin, within macrophages. Tanaka and colleagues examined serial sections of brain tissue from 3 patients with various causes of death (Tanaka et al., 1999). The sections were examined at the site of small hypointense lesions detected on a T2*-weighted echo plannar imaging (EPI) MRI undertaken prior to patients' death. They found 3 CMBs corresponding to haemosiderin pigments contained within macrophages around arteriosclerotic microvessels. Interestingly, one lesion also had an associated miliary pseudoaneurysm with a structure similar to that of 'bleeding globes' described previously (Matsuoka, 1939; Fisher, 1971b), which was connected to an arteriosclerotic vessel (Figure 26), while other vessels demonstrated the effects of hypertensive small artery disease with

fibrinoid necrosis. An additional finding of this study was the presence of a small infarction around the microaneurysm where macrophages had accumulated.

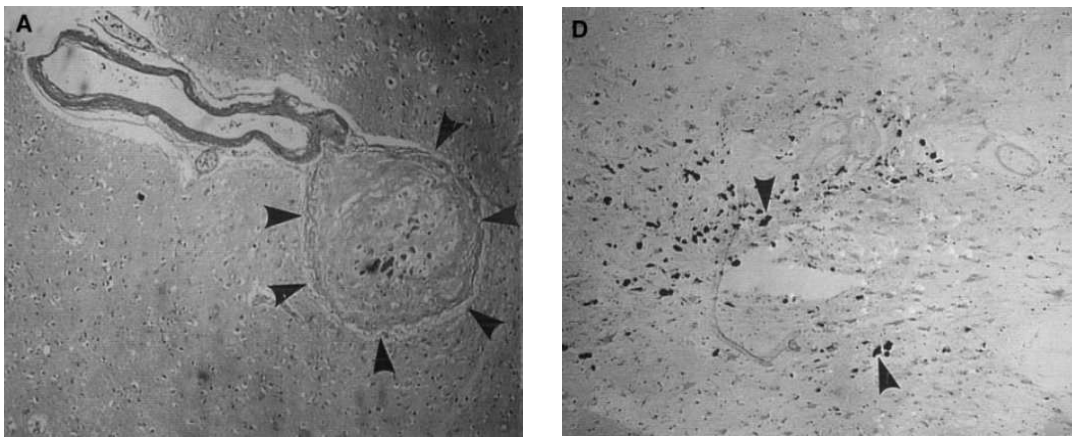


Figure 26. Pathological specimen from an autopsy case showing a miliary pseudoaneurysm and haemosiderin deposits around arteriosclerotic vessels.

A. shows an organised miliary pseudoaneurysm (arrows) connected to an arteriosclerotic microvessel. In **D**, haemosiderin pigments are contained within macrophages and are abundant around the arteriosclerotic microvessels.
From Tanaka et al., 1999

In the same year, Fazekas and colleagues undertook a histopathological analysis of MRI hypointensities of 11 patients who had died from ICH and confirmed that CMBs corresponded to collections of haemosiderin containing macrophages in the majority (61.8%) of cases (Fazekas et al., 1999) (Figure 27). Haemosiderin deposits were also noted without MR signal changes in two brains. The MRI-negative lesions (seen only on histopathological analysis) were smaller and consisted of only a few macrophages. In the CMBs without haemosiderin-laden macrophages, no specific pathology was found.

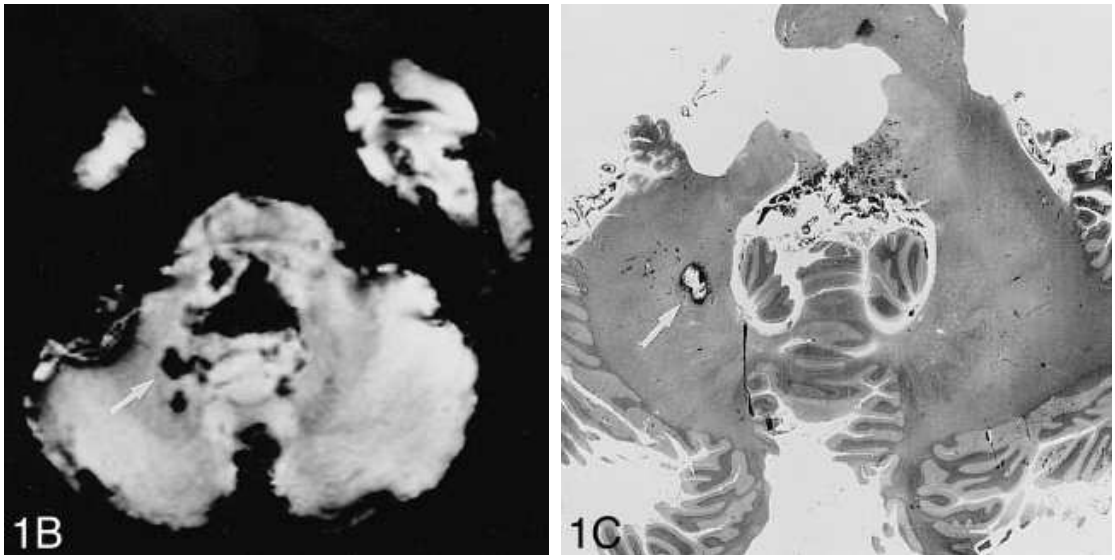


Figure 27. Histological-MRI correlation of CMBs.

1B. Post-mortem GRE T2*-weighted MRI showing foci of signal hypointensity in the cerebellum and pons. **1C**. A CMB corresponding to the largest hypointensity is visible on the corresponding histopathological section
From Fazekas et al., 1999.

In both studies, all specimens with hypointense lesions showed moderate to severe lipofibrohyalinosis of the adjacent arterioles, suggesting that these deposits result from the rupture of these arterioles (Fazekas et al., 1999; Han et al., 2009; Tanaka et al., 1999; Naka et al., 2004). Brains with fibrohyalinosis showed CMBs preferentially in the basal ganglia and thalami. It was also noted that haemosiderin deposits were surrounded by gliosis and incomplete ischaemic necrosis, therefore suggesting that systemic hypertension is associated with both ischaemic and haemorrhagic lesions.

In Fazekas' study, two types of vascular changes were observed (Fazekas et al., 1999). Lipofibrohyalinosis related to hypertension was one of them, and patients with pure lipofibrohyalinosis exhibited haemosiderin deposits in the deep regions (basal ganglia and thalami) followed by infratentorial regions. However, CMBs were also observed in cortico-subcortical regions in two specimens with additional CAA, the second type of vascular changes observed. These findings suggested a different distribution of CMBs according to the underlying arteriopathy. Further reports have confirmed a high frequency and preferential occurrence of cortico-subcortical CMBs in CAA (Greenberg, 2006; Schrag et al., 2010), whereas CMBs are usually found in the basal ganglia, deep white matter and brainstem in patients with chronic hypertension (Greenberg et al., 2009b; Tatsumi et al., 2008b) (Figure 28). In some cases, cortico-subcortical CMBs are found in patients with

hypertensive microangiopathy, suggesting that both arteriopathies can coexist in some patients causing an overlap in the distribution of CMBs.

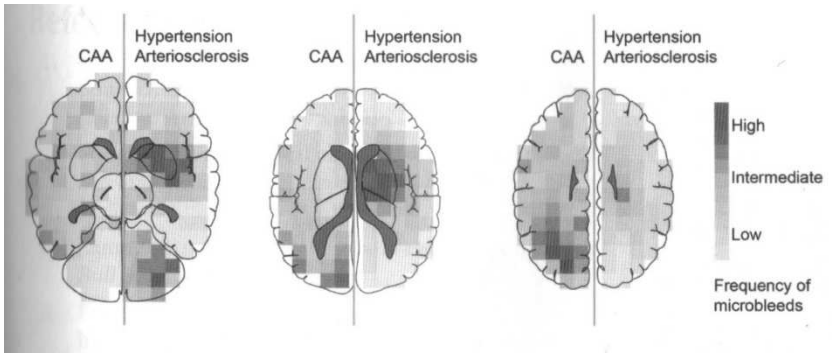


Figure 28. Illustration of the relative frequency of CMBs in CAA and in hypertensive arteriopathy.

The images represent different axial sections of the brain and the colours are coded to represent the likelihood of encountering CMBs in all brain regions with high likelihood (dark grey) and low likelihood (light grey). Data were compiled from selected MRI studies of patients clinically diagnosed with CAA or hypertensive arteriopathy (not on pathological confirmation).
From Werring, 2011

Subsequent histopathological studies of CMBs showed that the majority of lesions were small foci of haemorrhage composed of haemosiderin-laden macrophages at the site of radiological CMBs, in keeping with earlier studies.

In a post-mortem case report by Tatsumi et al. (2008b), almost all CMBs were found to be haemosiderin laden macrophages and were frequently associated with hypertensive microangiopathy. Schrag and colleagues reported 38 CMBs in 6 cases of AD in a histolopathology study combined with susceptibility-weighted imaging (SWI) (Schrag et al., 2010). Sixteen of the lesions were described as old haematomas which stained for iron, 10 lesions contained intact erythrocytes, 7 revealed cavitary lesions associated with a gliotic capsule containing haemosiderin granules and haemosiderin-laden macrophages, 3 contained haemosiderin granules and haematoidin depositon, 1 was a microaneurysm and another a dissected vessel. Most lesions contained evidence of CAA with vessel wall thickening, Aβ replacement of smooth muscle cells and microaneurysms.

In 2009, Greenberg and co-workers sought to explore the pathological changes that predispose to bleeding in patients with CAA (Greenberg et al., 2009b). The neuropathological analysis of six autopsied brains with high and low CMB counts showed that CAA subjects with many CMBs demonstrate significantly thicker amyloid-positive

vessels than those with few CMBs. This suggested that microbleeding may occur as a consequence of the increased wall thickness of amyloid positive vessels reflecting more severe CAA pathology. Vessel wall thickness might be a risk factor for microbleeding in CAA. However, the mechanisms determining the degree of wall thickness are unknown, and whether this mechanism holds true in hypertensive microangiopathy remains unclear.

MRI-histopathology correlations have also allowed further characterisation of the size of CMBs. Recent studies have systematically compared the size of CMBs on MRI with the actual diameter of the deposit assessed by histology. An autopsy imaging study of 38 lesions in 10 brains with CAA showed a range of 0.5-5 mm lesional (Schrag et al., 2010), much smaller than the size on MRI which was consistently overestimated ('blooming effect'). The magnitude of blooming appears greater for smaller lesions.

As of 2010, the literature had reported the examination of two normotensive and two hypertensive brains (Tanaka et al., 1999; Tatsumi et al., 2008b); 11 brains with spontaneous ICH (Fazekas et al., 1999); 16 CADASIL brains (Dichgans et al., 2002; Yamamoto et al., 2009); 19 brains with CAA (Greenberg et al., 2009a; Schrag et al., 2010) and 8 patients with vascular disease (Yamamoto et al., 2009), making a total of 58 brains. The findings of 10 such studies are summarised in Table 6. In 2011, Shoamanesh and co-authors published a systematic review of studies combining MRI and histopathology of CMBs (Shoamanesh et al., 2011). Only 5 studies met the inclusion criteria. From these, haemosiderin deposition was the most common histopathological correlate of CMBs, followed by old haematomas, no specific pathology, intact erythrocytes, vascular pseudocalcification, microaneurysm and dissected vessel. Some of the lesions contained erythrocytes, suggesting that not all CMBs are chronic in nature. In keeping with previous studies, lipofibrohyalinosis and CAA were the dominant vascular changes associated with CMBs (Shoamanesh et al., 2011). CMBs were cortico-subcortical among patients with documented CAA, in line with previous reports. Shoamanesh et al. also commented on the fact that in many studies, CMBs are often associated with a degree of surrounding tissue necrosis, which may be implicated in their clinical effects. One might be tempted from these studies to calculate the sensitivity and specificity of MRI for the detection of CMBs. This has been attempted in several reports (De Reuck et al., 2011; Shoamanesh et al., 2011). However, the small sample sizes, variation in the methods of detection and imaging parameters preclude a reliable estimation.

As these preliminary reports show, the number of CMBs detected depends on MRI characteristics, such as pulse sequence, sequence parameters, spatial resolution, magnetic field strength and image post-processing. This emphasizes the importance of taking into account the MRI technique used in the interpretation of study results, as we will discuss later in section 2.2, chapter 4 and chapter 9. Each of these factors has a potential effect on conspicuity and detection of CMBs and may influence the reported prevalence of CMBs. For example, increased magnetic field strength to 3 tesla or higher seems to improve CMB conspicuity. The use of image post-processing techniques (e.g. SWI) gives a higher contrast index between brain tissue and haemosiderin deposits that does conventional T2*-weighted MRI. The use of three-dimensional T2*-weighted MRI at high resolution can detect more CMBs than conventional two-dimensional GRE at lower resolution. We have shown how echo times affect the detection of CMBs in one of our papers (Gregoire SM, Clin Radiol 2009). However, limitations of MRI studies of CMBs are that they do not provide microscopical evidence for the specific tissue changes. Histopathology might be more sensitive for the detection of small vessel related changes, with the limitation that CMBs are small and diffuse in the brain and that examination of the entire brain in microscopic section is not possible. CMBs might be small enough to be fully contained within even a thin (e.g. 5 mm) brain slice, which prevents them from being visible to the examining pathologist. Future MRI developments, ie. ability to sample the whole brain at spatial resolutions less than 1 mm, may ultimately allow the detection of CMBs with higher sensitivity than histopathology. Further histo-pathological studies using these more advanced techniques will be required to assess the relative sensitivities of MRI versus pathology. Other limitations to the reporting of CMBs are the imaging analysis techniques and reporting methods which have varied substantially across studies. CMBs have many mimics which can be easily mistaken for CMBs by the inexperienced observer. The use of standardised instruments to rate their presence may improve identification reliability. The Microbleed Study Group published consensus criteria on the detection and reporting of CMBs including recommended criteria for identification of CMBs (Greenberg SM, Lancet Neurol 2009b). We also developed a rating scale for CMBs aiming at standardizing identification and reporting, that will be discussed in Chapter 4.

Table 7. Summary of publications correlating MRI findings and histology of CMBs as of 2009

Adapted from Werring, 2011.

	Number of patients (MRI)	MRI Seq (T)	Number of PM MRIs	Number of CMBs	Number of blocks (micro)	Histological techniques	Disease	Correlation /hypothesis	Results and Conclusions
Schrag et al., 2009	n/a	HR 3D GRE T2* SWI (3T)	10	38	13	H&E amyloid: Aβ Macrophages: CD68 Complement: C6, IHC Lymphocytes: CD3, CD20 Neurons: MAP2 Haemoxygenase ; HC ; Iron	CAA	CAA and CMB correlation PM MRI and PM histology Size correlation MRI-histology	• The vast majority of lesions located near the cortical ribbon: • 2 main areas: beneath the grey-white matter junction and in the superficial cortex where pial arterioles penetrate the grey matter • In several haemorrhages it was possible to determine which vessel ruptured. • One hypointensity was caused by a microaneurysm • SWI consistently overestimates the diameter of small lesions ('blooming effect')
Greenberg et al., 2009	46		6	181		H&E Aβ IHC	CAA	• Comparison high and low CMB count brains • Comparison micro vs macrobleed • Comparison CAA pathology with CMB frequency	• Increased wall thickness of amyloid-positive vessels in high CMB-count brains relative to the low CMB group • No other qualitative pathological differences (frequency or type of amyloid-positive vessels) • No correlation with dyshoric amyloid deposits, double barrel vessels • In vivo study: haemorrhage size in bimodal distribution (mixture of 2 separate populations)
Yamamoto et al., 2009	9 +co	T2 FLAIR	9+ co	n/a	>9	H&E; CV; LFB SMA; Coll IV GLUT1	CADASIL	• Pathological changes in WM of temporal pole in CADASIL; correlation to histology	• Small vessel arteriopathic and perivascular space widening • Perivascular space significantly widened in CADASIL compared to subcortical vascular ischaemic dementia
Tatsumi et al., 2008	1	GRE T2* SWI (3T)	1	9	9	H&E Iron	HTN	• Correlation MRI-histology • Case study	• All CMBs were haemosiderin deposits and frequently associated with hypertensive microangiopathy • Associated with lipohyalinosis or microaneurysms
Kikuta et al., 2007	1	GRE T2* SWI (1.5T)	n/a	1	1/1	H&E EvG Factor VIII IHC	Moya-Moya	• Hypointensity = CMB? • Case study	• MRI CMB confirmed by histology (small haematoma)
Dichgans et al., 2002 ; Messori and Salvolini, 2003	16 (+16 con)	GRE T2* 1.5T	7	94		H&E Iron	CADASIL	• Compare in vivo and PM imaging • Compare PM imaging with PM histology	• All CADASIL individuals show hyperintense T2-weighted abnormalities on brain MRI • Focal accumulations of haemosiderin-containing macrophages in 6/7 brains • Significant correlation between age and number of CMBs

Tanaka et al., 1999	89	FSE T2, T2* (1T)	3	108 (17 pts)		H&E MTC Myelin (KB)	HTN	• Compare in vivo and PM imaging • Compare PM imaging with PM histology	• Small hypointense lesions are foci of old haemorrhages, caused by rupture of arteriosclerotic microvessels • Incomplete ischemic necrosis in the surrounding areas • Old haemorrhages = haemosiderin pigments One case of organised pseudoaneurysm
Roob et al., 1999b		GRE T2* 1.5T	7	34	34	H&E; MTC Myelin (KB); Iron Amyloid (CR)	HTN	• Compare PM imaging with PM histology	• Most CMBs were haemosiderin deposits and frequently associated with hypertensive microangiopathy • No detection of pathology in 13/34 regions
Roob et al., 1999a	280	GRE T2* 1.5T	n/a	n/a	n/a	n/a	HTN	• Surveillance of elderly population (prevalence of CMBs)	• CMBs in 18/280 neurologically normal patients
Fazekas et al., 1999	n/a	GRE T2* (1.5T)	11	34	11	H&E; MTC Myelin (KB) Iron Amyloid (CR)	HTN	• Hypointensity = CMB = histology?	• All brains examined showed moderate to severe small-vessel disease • Areas of signal loss predominantly in basal ganglia, thalami, brain stem, or cerebellum

2.1.4 The link between haemosiderin deposits and cerebral microbleeds

As discussed above, MRI-pathology correlations have shown that the majority of radiologically detected CMBs relate to small foci of haemosiderin deposits in the vicinity of pathologically damaged small vessels, in the context of widespread small vessel vasculopathy, typically hypertensive arteriopathy or CAA. These may occasionally be related to aneurysmal or pseudoaneurysmal lesions rather than well-defined extra-mural bleeds. The alteration of the walls of small vessels may theoretically produce steady extravasations of erythrocytes through the fragile vascular walls (Roob and Fazekas, 2000) or tiny areas of frank haemorrhage (Werring, 2007). However, this mechanism as a cause for CMBs remains speculative. MRI-pathology correlations are limited by technical factors which hamper the understanding of the causes of CMBs. Firstly, CMBs are small (radiologically <5 mm, and pathologically often much smaller than this) and widespread in the brain, and can easily be missed by visual inspection of post-mortem material; their identification requires careful and systematic examination of pathology specimens. Secondly, correlation studies are descriptive and unable to establish a causal relationship between the presence of vascular changes and of CMBs (Brandner, 2011) These technical aspects probably account for the small number of brains with CMBs that have been analysed in correlation studies.

To date, the pathological and radiological relationship between the haemosiderin foci and radiologically-defined CMBs is not yet resolved. As discussed, the common view is that CMBs arise from extravasation of red blood cells, resulting in pericyte erythrophagocytosis, haemoglobin degradation and haemosiderin deposition. However, this hypothesis was recently challenged. Alternative explanations to the origin of haemosiderin deposits have been proposed based on evidence from CADASIL and peripheral injury. Perivascular haemosiderin deposits are observed in CADASIL, a condition associated with severe vascular fibrosis and no propensity for haemorrhage. Skin examination in the context of chronic venous stasis of the leg shows perivascular deposits to be infrequent (Caggiati et al., 2008). The hypothesis made by the group led by P. Ince is that haemosiderin deposits may come from local iron sources within the brain, as opposed to haemoglobin degradation (Janaway et al., 2013). Iron within the brain is normally stored within oligodendroglial cells, especially in the basal ganglia (Morris et al., 1992). The damage to tissue caused by small vessel disease related ischaemia causes release of the iron stores from oligodendroglial cells, which exceeds the ability of the surrounding tissues to process

it. The local excess of iron could therefore be processed by macrophages to haemosiderin and result in perivascular accumulation. This hypothesis was tested in a population-based neuropathology study correlated with post-mortem 3T MRI (Janaway et al., 2013). In the study, greater putamen haemosiderin was significantly associated with age, markers of small vessel disease related ischaemia (microinfarcts, arteriosclerosis and lacunes) and with widespread CMBs, but not with measures of neurodegeneration. This suggests that the ageing brain becomes more vulnerable to excessive release of iron from ischaemic damage as the number of healthy cells able to process it (e.g. oligodendroglial cells) decreases. CMBs may therefore also be a marker of focal haemosiderin deposition secondary to parenchymal ischaemic damage. Furthermore, almost all patients (aged 65 and over) had focal haemosiderin deposits, suggesting that histology is more sensitive for detecting haemosiderin in post-mortem brain tissue than MRI analysis, and that their size needs to be sufficient to be able to detect them on MRI. Other studies confirmed that the overwhelming majority of subjects above the age of 70 have haemorrhages at the capillary level (Cullen et al., 2005; Fisher et al., 2010). The presence of CMBs in the frontal lobe white matter in patients with high amount of haemosiderin deposits in the basal ganglia further suggests that CMBs reflect widespread small vessel disease.

To conclude, the literature on CMBs runs to less than 100 brains to allow the determination of what the hypointensities observed on MRI mean. From these studies, there is no doubt that CMBs correspond to pathology-defined haemosiderin deposits in the perivascular regions. They are very common in the ageing brain, but only those of sufficient size, i.e. in aggregates, will be detectable by MRI. From their association with small vessel arteriopathies (hypertensive and CAA), it is clear that their presence may be a valuable indicator of the presence and severity of cerebral small vessel disease. The latest research suggests that their origin and implications might differ according to their distribution in the brain. In the cortex, CMBs likely reflect the presence of CAA, a condition associated with perivascular bleeds, microaneurysm formation and lobar haemorrhages (Maia et al., 2007). Consequently, in CAA, it is likely that lobar CMBs are a biomarker for the risk of ICH. By contrast, in the deep brain, the origins of CMBs are less clear, and may result from either perivascular bleeding from damaged vessels, or from release of parenchymal iron stocks following ischaemia. Therefore the relevance of deep CMBs as a marker for the risk of ICH remains to be determined. Further large, well-designed histopathological studies combined with MRI correlation are needed to characterise the range and threshold of haemosiderin

pathology required to create a CMB on MRI and confirm the bleeding or ischaemic origin of CMBs according to the underlying arteriopathy.

2.2 Detection of cerebral microbleeds using magnetic resonance imaging

2.2.1 Introduction to the principles of magnetic resonance imaging

Basic principles

Since this thesis contains a number of studies relating to MRI findings, an understanding of the basic principles of MRI is necessary. MRI is a medical imaging technique based on nuclear magnetic resonance (NMR) principles that uses the intrinsic magnetic properties of the atoms within tissues and some extrinsic parameters to produce sophisticated images of the human body (Faulkner, 1996). In contrast to CT, it does not involve ionising radiation. It uses a powerful magnetic field to align the nuclear magnetisation of hydrogen atoms in the body tissues (Lauterbur, 1973) and provides a much greater contrast between soft tissues than CT.

NMR describes the application of a pulse of radiofrequency (RF) energy to a sample in the presence of a magnetic field gradient, followed by the collection of a RF signal emitted by the sample (Werring, 2001). The RF signal depends on the interactions that occur when atomic nuclei are placed in an external magnetic field. Specifically, all tissues are composed of atoms, and the nuclei of these atoms contain protons. Those nuclei with an odd nuclear number (protons + neutrons = odd number) have two important intrinsic properties: first, they have a special property to spin and second, because they are charged, they create a local magnetic field. Each nucleus behaves like a magnetic dipole with a magnetic field. The most important such atom in MRI is the hydrogen nucleus (which contains a single proton), contained in water molecules of biological tissues. Each spinning proton may be represented as a vector showing the direction of its magnetic moment (Figure 29).

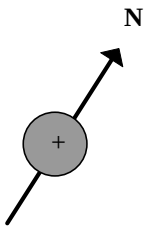


Figure 29. Schematic representation of a proton.

Protons are charged particles which have the property of spin and have a magnetic moment which may be represented by a vector.

In a resting state there is no net magnetisation because the sum of all the spin vectors of protons is zero (Figure 30). However, when the tissue is placed in an external magnetic field (conventionally designated B_0), these protons absorb or emit energy and realign themselves, and a net magnetisation is produced along the longitudinal axis of the magnetic field. As the majority of protons adopt the parallel (lower energy) state, the subject in the scanner is magnetised with a small net magnetic moment (M_0). This magnetic moment is also called longitudinal magnetisation, and is directly proportional to B_0. In clinical scanners, the magnetic field is commonly 1.5T (Tesla) in strength and is orientated horizontally.

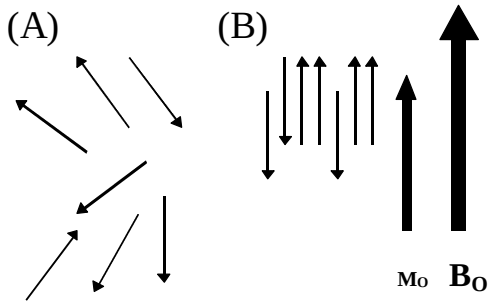


Figure 30. The proton magnetic moments ('spins')

(A) Proton magnetic moments are normally oriented randomly. **(B)** When placed in an external field (B_0), they become aligned in a parallel or antiparallel state causing them to acquire a net magnetic moment, M_0.

Protons placed in a magnetic field exhibit a type of motion called 'precession'. The precession can be conceptualised as a vector representing the magnetic dipole describing the shape of a pole (Figure 31).

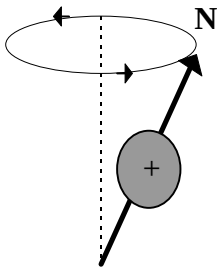


Figure 31. Precession of a proton in an external magnetic field.

The precession frequency, designated by ω, depends on the strength of the applied magnetic field (B_0 given in Tesla) and the molecular species under consideration, and is characterised by a gyromagnetic ratio (γ), according to the Larmor equation:

$$\omega = \gamma B_0$$

In clinical MRI, the magnetic moment is manipulated to produce MR images. The simplest manipulation is the application of a short burst of energy, called a RF pulse (usually a 90^o pulse). RF pulses are short duration oscillating magnetic fields with a specific frequency that causes the protons to produce a rotating magnetic field (they 'resonate'). The frequency required to alter the alignment of the magnetisation is directly related to the precession (Larmor) frequency, which for protons in a 1.5T magnetic field is approximately 64 MHz (Werring, 2001). When the RF pulse is applied the precessing protons undergo re-arrangement in two ways: firstly, some protons will gain energy and become realigned in the higher energy anti-parallel state; and second, the protons will begin to precess in a more coherent way (Figure 32). The former effect induces a reduction in the longitudinal magnetisation of the sample, as they are less parallel orientated protons. The latter effect causes the sample to acquire a transverse orientated magnetic moment, which is rotatory due to the in-phase precession of the protons. These alignment changes generate a signal which can be detected by the scanner and manipulated by additional magnetic fields to provide more information and construct an image of the body.

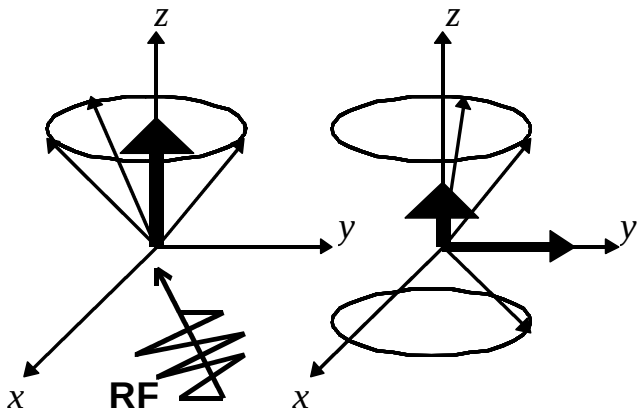


Figure 32. Application of a radiofrequency pulse

When a RF pulse is applied at the appropriate frequency (left) protons lose their longitudinal magnetisation and gain transverse magnetisation (right).

In this process, the protons are 'tipped' from their previously longitudinal alignment by an angle, α. If this angle is 90°, then the RF pulse is commonly termed a '90° pulse'. The larger the amplitude and the longer the duration of the RF pulse, the larger the angle. NMR measurements are made from the signal induced by the transverse magnetisation caused by the RF pulse. Because transverse magnetisation is an oscillating magnetic field, it can induce an electrical current in an appropriately placed receiver coil (Figure 33). The point for creating an MR image starts from when the RF pulse is switched off because this causes signal changes.

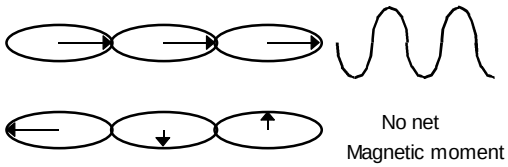


Figure 33. The net oscillating magnetic field produced by protons precessing in phase.

This net oscillating magnetic field can induce a signal in a receiver coil that can then be detected.

When the RF pulse stops, the protons release the absorbed energy and immediately try to return to their equilibrium position, a process known as relaxation (Brown and Semelka, 2003). The longitudinal magnetisation will increase back to its original size, M_0, and the newly acquired transverse magnetisation will diphase due to slight differences in the precession rates of individual protons (Figure 34). The process of relaxation is described by two time constants, T1 and T2, corresponding to the longitudinal and transverse relaxation, respectively.

After the application of RF, the longitudinal magnetisation is lost, and the magnetisation of the tissue is in the transverse plane. When the RF stops, the time required for protons to achieve longitudinal magnetisation again (63% of its original value) by releasing the absorbed energy is the T1 relaxation time. The released energy is transferred to its surroundings (the 'lattice'); therefore, the longitudinal relaxation is also referred to as *spin-lattice relaxation.* In comparison, transverse relaxation occurs because each proton experiences a slightly different magnetic field (due to inhomogeneities in the B_0 field and to interactions with surrounding protons), causing each one to naturally precess at a different frequency. The net result is that when the RF pulse is switched off, protons lose their phase coherence and the transverse magnetisation decays. The time required for the transverse magnetisation to decay to 37% of its initial value and is the T2 relaxation time. The released energy is transferred from an excited proton to a neighbouring proton; therefore, the transverse relaxation is called *spin-spin relaxation*. If efforts are made to minimise the external field inhomogeneities, then transverse relaxation is described by the constant T2, and if inhomogeneities are present, then the dephasing will be more rapid, and the process is described by a shorter constant termed T2* ('T2 star'). Because dephasing is a very rapid process, longitudinal relaxation (T1) takes much longer than transverse relaxation (T2) (Brown and Semelka, 2003).

Diseased tissues can be identified on MRI because the protons in their tissues return to their equilibrium at different rates. This is due to the physicochemical environment which is different in different tissue types and is affected by pathological changes. Protons that are tightly bound within large molecules such as lipids, proteins or carbohydrates, will not be 'seen' on conventional scans because they will lose their magnetisation to surrounding molecules very quickly (i.e. they have a short T2). The unbound, 'free' water protons (with a long T2), both intracellular and extracellular, are the protons whose movements are detected by MRI.

The Spin Echo experiment

The spin echo (SE) sequence is the most commonly applied sequence in neuroradiology (Tress and Brant-Zawadski, 1985). It can be used to image tissues with different T1 and T2 relaxation properties resulting in T1-weighted and T2-weighted images and uses two RF pulses. First, a 90° pulse is applied to create the detectable oscillating transverse magnetisation which induces a signal in a receiver coil. This signal is rapidly lost due to dephasing as a result of T2* decay. Next, a 180° pulse, called the *refocusing pulse,* is applied and this refocuses the lost phase coherence. This pulse tips the protons by 180° in the transverse plane. This has the effect of speeding up the protons which are precessing more slowly, allowing them to 'catch up' with the faster precessing ones (Figure 34). At this stage, protons again precess coherently, and this generates an oscillating magnetic field, detectable by a receiver. The rephasing of protons and the detection of the signal is termed the *echo* or *echo collection*.

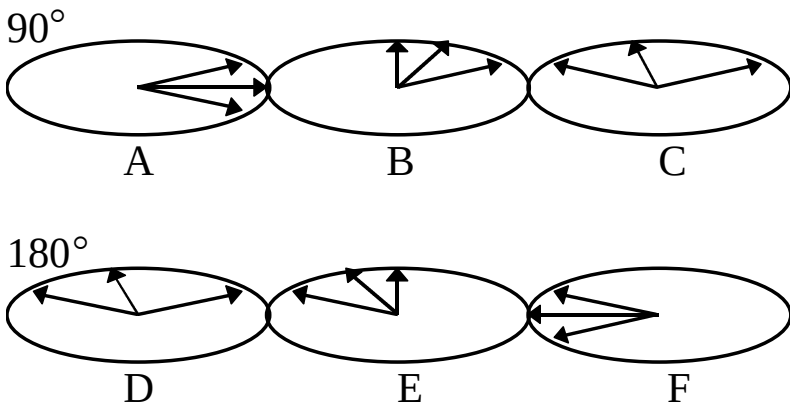


Figure 34. Applications of the 90° and 180° RF pulses

After the RF pulse is switched off the protons diphase (A, B, C; top). The 180° pulse reverses their precession directions, restoring phase coherence (D, E, F; bottom).

The time to echo (TE) is the time between the application of the 90° pulse and the peak of the detected signal (Jackson et al., 1997; Scherzinger and Hendee, 1985). To create an image, the experiment must be repeated many times; therefore the 90° and 180° pulses are repetitively applied. The time between consecutive applications of the 90° pulse is the time to repeat (TR). The SE sequence may be depicted by a pulse sequence diagram, as shown in Figure 35.

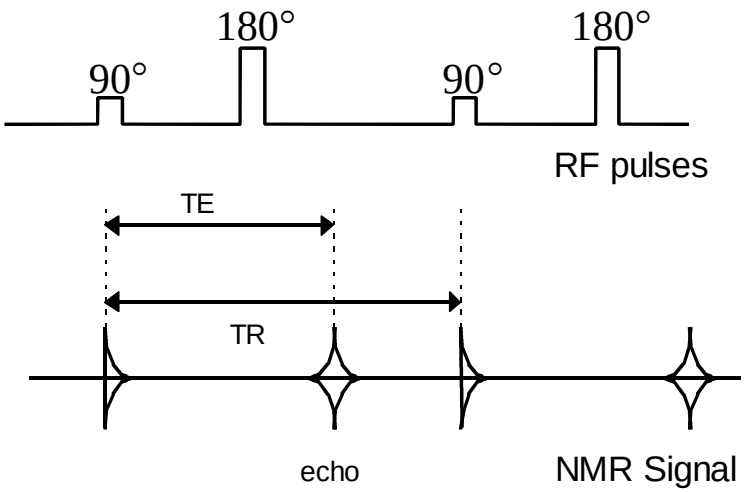


Figure 35. Pulse sequence diagram for a simple spin echo NMR experiment.

Consequently, TE and TR determine the resulting image contrast depending upon the differences in T1 and T2 relaxation times (Jones et al., 1992). If a long TE is used, at the time of echo collection protons with a short T2 will have dephased, whereas those with a longer T2 (i.e. longer than the TE) will not. Therefore, tissues with a short T2 will return little NMR signal and appear dark on T2-weighted images, whilst those with a longer T2 will return a high signal and appear bright. If a T1-weighted image is required, then a short TR is chosen, meaning that tissues with a long T1 will not have had the time to regain their original longitudinal magnetisation, and will give a smaller NMR signal following their next excitation. If the T1 is short, the signal is high (bright on the image); if the T1 is long, then the signal is low (dark on the image).

The T1 and T2 properties of protons tend to vary together. In biological tissues, T1 is usually of the order of 300 to 2000 ms, and T2 of the order of 30 to 150 ms. Tissues with very mobile protons have longer relaxation times; e.g. CSF has a long T1 and a long T2. More structured tissues have a shorter T1 and T2 because they contain less mobile protons that readily exchange energy with their surroundings. Pathology usually increases the water content in tissues and the mobility of protons; this causes lengthening of the T1 and T2 of healthy surrounding tissues.

Creating an MR image

In order to create a meaningful image, additional information regarding the spatial location of the NMR signals into the three dimensional space is required. To do this, magnetic field gradients that convert information about physical position into frequency data are briefly

applied during the imaging sequence in order to localise the signal coming from each voxel of tissue. These can induce protons in different positions along them to have different precession frequencies. Three types of gradients are applied along the three orthogonal axes of the scanner magnet coordinate system. These gradients are termed the slice-selection (z axis), frequency-encoding (x axis) and phase-encoding (y axis) gradients.

The slice-selection gradient allows slices of tissues to be selectively excited, depending on the range of frequencies of the RF pulse applied. This gradient is then switched off, and the other two gradients are then applied. These latter gradients will help to determine where the point on the slice from which the signal arises is located by altering this signal. The frequency-encoding gradient is applied in the x axis direction resulting in different precession frequencies along this axis. Each resulting signal of different frequencies contributes to the net NMR signal emitted by the slice. After the frequency-encoding gradient, a third y axis gradient, called the phase-encoding gradient, is very briefly applied to finally localise a given tissue volume element. This results in a different precession frequency along the y axis. The signal created by the dephasing of the protons in their new y axis positions will be collected when this gradient is switched off. It should be noted that the frequency- and phase-encoding gradients may each be applied to either the x or y axis.

The spatial resolution is determined by a factor called the gradient-time product, or κ. This corresponds to the achievable spatial resolution of the image and depends on the gradient amplitude (G) and the time for which it is applied according to the equation:

$$K = Gt$$

This is because larger field gradients enable spins that are closer together to be differentiated. Therefore, the larger the field gradient, the smaller the image features received and the higher the resolution. Using digital sampling, a two-dimensional κ space matrix is created from the collection of the NMR signal at different time points on the x and y axes.

The final MR image will therefore depend on two types of factors; the intrinsic parameters (that are related to the tissue) and the extrinsic parameters (related to the scanner and set by the physician or technologist). Changing the parameters of the scanner allows the creation of contrast between tissues and allows them to be distinguished. It creates images utilising the extrinsic scanning parameters (TE, TR, field of view, slice thickness, slice gap

and resolution), and the intrinsic (physical and chemical) properties of atoms within tissues (including spin angular moment, local magnetic field, spin-lattice relaxation and spin-spin relaxation) (Jackson et al., 1997; Tress and Brant-Zawadski, 1985). Based on these parameters, pulse sequences are selected which correspond to the manner in which the RF pulses. A pulse sequence defines the manner in which the RF pulses and magnetic gradient fields are applied (Jackson et al., 1997; Scherzinger and Hendee, 1985).

Echo planar imaging

In conventional SE, repetitive excitations are performed and echo collections are successively performed to obtain an MR image. Echo planar imaging (EPI) is a technique that was proposed to overcome the need for repetitive excitation pulses and to shorten the time of image acquisition. This technique allows image data to be collected from a single excitation pulse with an infinite TR. During this single excitation, the EPI collects the whole of the κ matrix because it traverses the κ space rapidly (in the time taken for the NMR signal to decay). For optimal resolution, large gradients in each spatial dimension are required as these determine the gradient-time product.

However, rapidity is underpinned by serious disadvantages; mainly the vulnerability to certain types of image artefact and distortion, especially at interfaces between tissue types (e.g. air and bone), causing a *magnetic susceptibility artefact*. This results in signal dropout and a geometric distortion of the image. The susceptibility artefact with specific reference to CMBs and CMB mimics will be discussed in the next paragraphs.

Gradient-recalled echo principles

An important aspect of MR is that the magnetic field is never 100% homogeneous. This inhomogeneity may arise from the main scanner magnetic field inhomogeneity (due to nearby building walls and metals) and tissue-induced non-uniformity.

When a tissue is placed in a magnetic field, its different parts behave differently because of their intrinsic paramagnetic properties. A paramagnetic effect on the local magnetic field is called *magnetic susceptibility*. Interfaces between soft tissues, e.g. bone and air (which have different magnetic susceptibility properties), also make the applied magnetic field inhomogeneous (Roberts and Mikulis, 2007). The problem of susceptibility can be solved by applying a second redirecting or refocusing RF pulse (Brown and Semelka, 2003). In cases where the second redirecting RF pulse is not applied, ongoing dephasing of the protons

occurs and their vectors remain incompletely aligned. This results in low signal on MRI (Brown and Semelka, 2003; Rajan, 1998).

Certain tissues, e.g. blood break down products (haemosiderin, deoxyhaemoglobin and methemoglobin) have paramagnetic properties (positive magnetic susceptibility) and when they are imaged by MRI, augmented spin dephasing and signal loss is observed. This effect causes these tissues to appear as larger areas of signal loss on MR images than the original size of the paramagnetic material, and is called the blooming or T2* effect (Koennecke, 2006; Roberts and Mikulis, 2007; Viswanathan and Chabriat, 2006). When imaging CMBs, this T2* effect is critical, since they are only seen due to their susceptibility effect.

A gradient-recalled echo (GRE) sequence is a pulse sequence significantly faster than SE sequences. As mentioned above, there is no 180° pulse (the refocusing pulse) and the single RF pulse is less than 90°. This reduces the scan time, but increases the signal-to-noise ratio. As the TE is increased, more artefacts are generated and signal loss becomes more prominent (Jackson et al., 1997). The images obtained are termed as T2*.

Due to the absence of a redirecting RF pulse, the susceptibility-related signal loss increases because of the field inhomogeniety, i.e. the T2* effect is emphasised, as discussed earlier. Thus, this sequence positively exploits the magnetic field inhomogeneities. This type of imaging protocol is termed a T2*-weighted GRE sequence (Brown and Semelka, 2003; Jackson et al., 1997; Rajan, 1998; Scherzinger and Hendee, 1985) and is used in a variety of clinical implications where paramagnetic blood breakdown products are to be detected (Jackson et al., 1997; Roberts and Mikulis, 2007; Scherzinger and Hendee, 1985).

T2* GRE sequences are very sensitive to the inhomogeneity of the magnetic field produced by the tissues. Each tissue has a specific way to respond to a magnetic field called the magnetic susceptibility. At the interface between tissues, the inhomogeneity caused by the magnetic susceptibility of the different tissues (soft tissues, bone, air) results in a local disparity causing signal loss. This signal loss is called the magnetic susceptibility effect (fast decay of the local MRI signal). Thus, when GRE T2* is applied to a tissue containing materials with paramagnetic properties, signal loss can be detected. Based on these properties, GRE T2* has a wide range of clinical applications (Roberts and Mikulis, 2007).

2.2.2 Detection of cerebral microbleeds

Signal loss with cerebral microbleeds

The types of sequences used and tissue magnetic properties such as relaxation times and proton density result in varying contrasts in MR images. Relaxation times are transverse (T2) and longitudinal (T1), and vary for different types of tissue. Theoretically, tissues with a long T1 will appear as a low signal on T1-weighted images and tissues with a long T2 will show as bright on T2-weighted images. Two factors influence the relaxation times of a tissue: the distribution of water within the tissue, and the surroundings. Among the surroundings, paramagnetic substances such as metals, have a strong effect on the relaxation times; they form a region of magnetic field inhomogeneity surrounding the paramagnetic centre (Stankiewicz et al., 2007). One of the metals that is commonly present in the human brain is iron, where it exists in the form of ferritin and haemosiderin and can be visualised by MRI because it is present in sufficient quantity and in the right oxidation state (Schenck, 2003; Schenck and Zimmerman, 2004). Histopathological studies have demonstrated that CMBs contain haemosiderin deposits (Schrag et al., 2010; Tatsumi et al., 2008a), a paramagnetic substance (Schenck and Zimmerman, 2004). Therefore, CMBs will cause a decrease in the T1 and T2 relaxation times.

Sequences sensitive for the detection of cerebral microbleeds

MRI provides a unique opportunity to detect blood residues of ICH throughout life. CMBs are composed of blood-breakdown products (haemosiderin, deoxyhaemoglobin and methemoglobin) which are paramagnetic and hence have considerable internal magnetisation when exposed to an MRI field (Greenberg et al., 2009b). This internal magnetisation generates local inhomogeneity in the magnetic field that surrounds the brain CMB and causes the susceptibility effect described above and concurrent signal loss (Brown and Semelka, 2003). FSE pulse sequences had a relative lack of sensitivity to magnetic susceptibility effects. T2*-weighted gradient recalled-echo (GRE) MRI enhances the magnetic susceptibility (and resultant signal dropout) caused by haemosiderin, thus increasing the sensitivity for haemorrhages (Atlas et al., 1988). The sequence has a high sensitivity for differences in magnetic susceptibility, and shows a higher sensitivity than conventional or FSE T2-weighted imaging for detecting previous small areas of bleeding (Greenberg et al., 1996; Roob et al., 1999b; Chan et al., 1996). Because haemosiderin remains stored at the site of a haemorrhage for many years, T2* GRE MRI is suitable to

assess the presence of past haemorrhages and is valuable in detecting chronic and small haemorrhages (Atlas et al., 1988; Offenbacher et al., 1996; Schenck and Zimmerman, 2004). Nowadays, iron-sensitive MRI sequences (mainly GRE T2* MRI and susceptibility weighted imaging [SWI]) are used to detect CMBs. As GRE T2* MRI has a greater sensitivity to susceptibility variations than SE and FSE techniques (Atlas et al., 1988), it is commonly used to detect blood-breakdown products resulting from haemorrhages and CMBs, and using a 1.5T field strength, it is the most commonly used technique. However, technical developments in this field are rapid, and it is likely that in the future, higher field strengths, thinner slices, and newer sequences (including SWI) will make CMBs a very common finding, especially in the elderly.

Because the blooming effect that characterises CMBs differs markedly with different MRI parameters (field strength, slice thickness, TE, interslice gap, TR, flip angle [FA], and matrix size), CMB detection may depend upon the specific MRI sequence used. Consequently, longer TEs (Tatsumi et al., 2008a), increased magnetic field strength (3T versus 1.5T) (Scheid et al., 2007), and new techniques such as thin section SWI (Nandigam et al., 2009) enlarge the susceptibility effect and, hence, may increase the size and number of CMBs detected compared to standard GRE T2* MRI sequences.

Susceptibility weighted imaging

SWI differs from GRE T2* images in that high resolution 3D gradient-recalled echo sequence with a long echo time utilising both magnitude and phase are created (Sehgal et al., 2005). These features make SWI more sensitive to detect CMBs than conventional gradient-echo T2* sequences (Nandigam et al., 2009), and currently SWI is the most sensitive technique to visualise CMBs in vivo (Ayaz et al., 2010). SWI can 'magnify' the size of the smallest bleeds (<1mm) up to three-fold (Schrag et al., 2010), and up to four times as many CMBs can be detected by SWI compared to conventional GRE T2* MRI (Nandigam et al., 2009).

The MR signal after Fourier transform is a complex signal resulting in a formation of real and imaginary images (Charidimou et al., 2013b). The combined real and imaginary parts form the magnitude images, which are used in conventional MR images. Also derived after the Fourier transform are phase images, which traditionally have been discarded as they are sensitive to both local field inhomogeneities as well as unwanted magnetic field inhomogeneities (Haacke et al., 2011) (Figure 36). By using high-pass filtering techniques to

remove large-scale inhomogeneities, the utility of the phase images is increased (Haacke et al., 2004). Important information is contained within the phase images, including differences in magnetic field strength in a particular voxel compared to the overall magnetic field. Therefore, the combination of both the phase and magnitude images into a single susceptibility-weighted image can be used to demonstrate blood products better than using magnitude images alone (Ayaz et al., 2010).

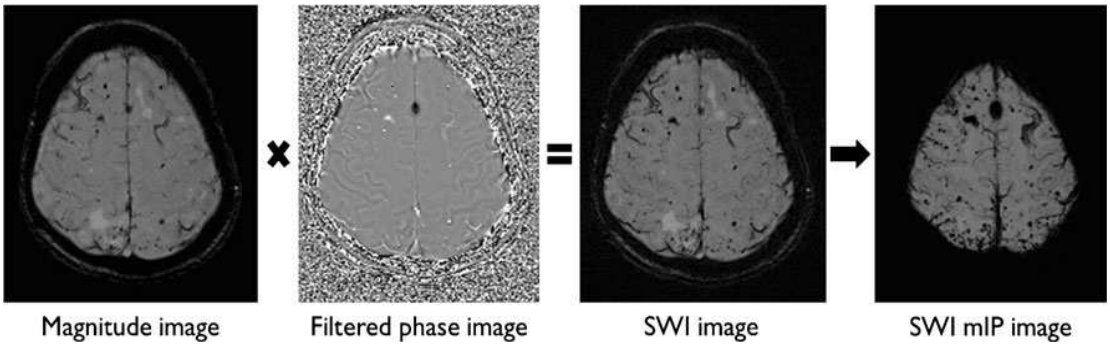


Figure 36. Post-processing steps to create a susceptibility-weighted image (SWI) in an elderly patient with CAA.

Thin section magnitude images are multiplied by unwrapped phase images (usually to the power of 4) to produce susceptibility-weighted images. This process increases the contrast between paramagnetic substances such as CMBs and surrounding tissue. The visibility of CMBs and cerebral veins (which contain paramagnetic deoxyhaemoglobin) is further enhanced by the generation of minimal intensity projection (mIP) images. The final image shows multiple strictly lobar CMBs and cortical superficial siderosis.
From Charidimou et al., 2013b

An added advantage of using the phase information is the ability to differentiate between blood products, which are paramagnetic, and calcification or mineralisation, which are diamagnetic (Charidimou et al., 2013b). On SWI-filtered phase images, calcium carbonate appears with the opposite-sign phase compared to haemosiderin (Wu et al., 2009). A further component of SWI that aids in differentiating between CMBs and their mimics is the use of minimum intensity projection (mIP) images. These images are taken over a number of sections and so help to discriminate between CMBs and blood vessels, as they are able to assess vessel continuity (Haacke et al., 2011).

Defining cerebral microbleeds: rationale

CMBs are an increasingly common radiological finding in stroke, neurological and general medical practice. Finding CMBs in the brain can raise a number of clinical questions regarding the safety of antithrombotic drugs, the risk of recurrent symptomatic ICH,

cognitive decline and clinical deterioration. These risks may depend on the presence of CMBs, but also their number and their anatomical distribution (e.g. lobar versus deep) in the brain. These questions are of clinical relevance, particularly as CMBs may be helpful in diagnosing small-vessel arteriopathies (including CAA) non-invasively in life. In order to tackle these questions, it is important to be able to reliably define and map CMBs in the brain. This may be challenging because of the many other lesions seen on MRI with similar morphological or signal characteristics (CMBs mimics, discussed in more detail in section 2.2.3).

Although there has been general agreement on the radiological properties of CMBs, there has until recently been a lack of standardised specific criteria with which to define them. Many research units have used in-house CMB rating methods, and although reliability (intra- and inter-observer agreement) has been reported, the exact methods used (detailed CMB definition criteria, anatomical boundaries, etc.) have not usually been fully described. A standardised approach with clearly described criteria for CMBs and their anatomical location may be helpful in improving reliability and allowing results from different centres to be compared. To date, there are two published CMB rating scales that have been validated in hospital cohorts of stroke patients. One of these scales was developed as part of this thesis (see Chapter 4). The radiological criteria for defining CMBs and an introduction to these standardised rating scales will now follow. The potential for automatically detecting and mapping CMBs in the future is discussed briefly at the end of the MRI section on CMBs.

How should cerebral microbleeds be defined?

Since the emergence of MRI techniques allowing the detection of CMBs, definitions of the MRI criteria for identifying CMBs have varied. However, all studies have used features relating to shape (described variously as rounded, dot-like, ovoid, spherical), size (small) and signal characteristics on MRI sensitive to susceptibility effects (dark or black, and of homogeneous signal). Using these criteria, most radiological lesions defined as CMBs do correspond to haemosiderin deposition in relation to pathologically abnormal small vessels (Fazekas et al., 1999; Schrag et al., 2010), sometimes related to microaneurysms (see section 2.1). Although many papers have described the various radiological features used to distinguish CMBs, the first consensus criteria for their identification were not published until 2009 (Greenberg et al., 2009a). On appropriate MRI sequences (typically GRE T2*or SWI) CMBs are defined as round or ovoid (rather than linear or curvilinear) foci of

homogeneous signal intensity loss. Irregularly shaped areas (e.g. linear, curvilinear, star-like) of signal loss or hypointense lesions with a diameter greater than 10 mm are considered old scars of ICH or 'haemorrhagic lacunes'. This definition has the purpose of excluding larger ICHs ('macrobleeds'), subarachnoid blood or siderosis, specific secondary causes of bleeding (e.g. arteriovenous malformations or tumours) and non-haemorrhagic causes of low signal (e.g. mineralisation, regions of air-bone susceptibility-related signal loss). These and other common mimics of CMBs are considered further in this chapter. Examples of typical CMBs are shown in Figure 37 and Figure 38.

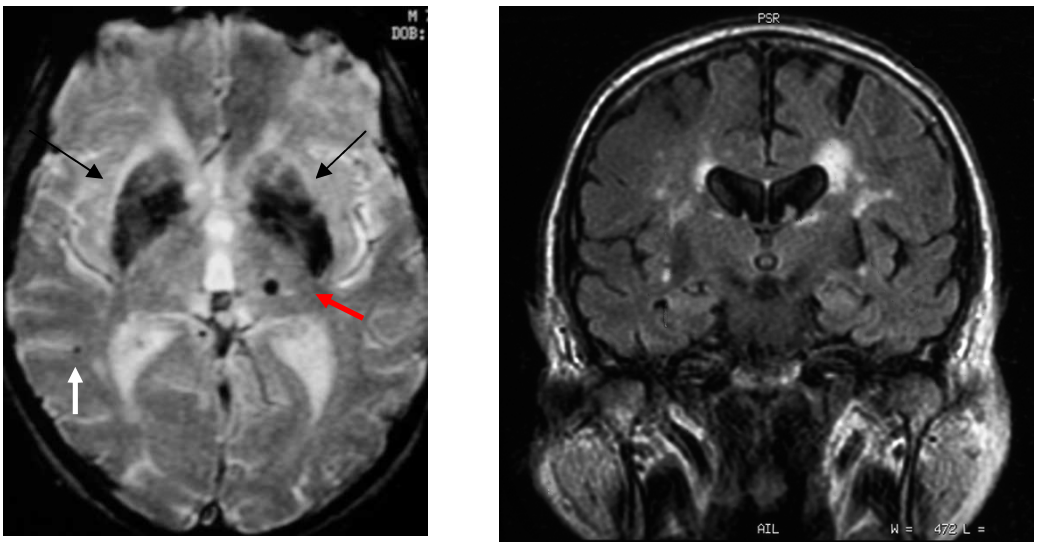


Figure 37. MRI example of a CMB (1)

GRE T2*-weighted MRI (left panel) and FLAIR sequence (right panel) of a 78 year old hypertensive man who developed a sudden onset of weakness in the right side of his face, arm and leg. The GRE T2*-weighted MRI shows a CMB (red arrow) in the left thalamus and several CMB mimics: vessel at the bottom of the sulcus (white arrow) and hypointensities in the basal ganglia due to calcification or iron deposition (black arrows). The FLAIR sequence (right panel) shows extensive white matter disease and areas of previous infarction (but no CMBs).

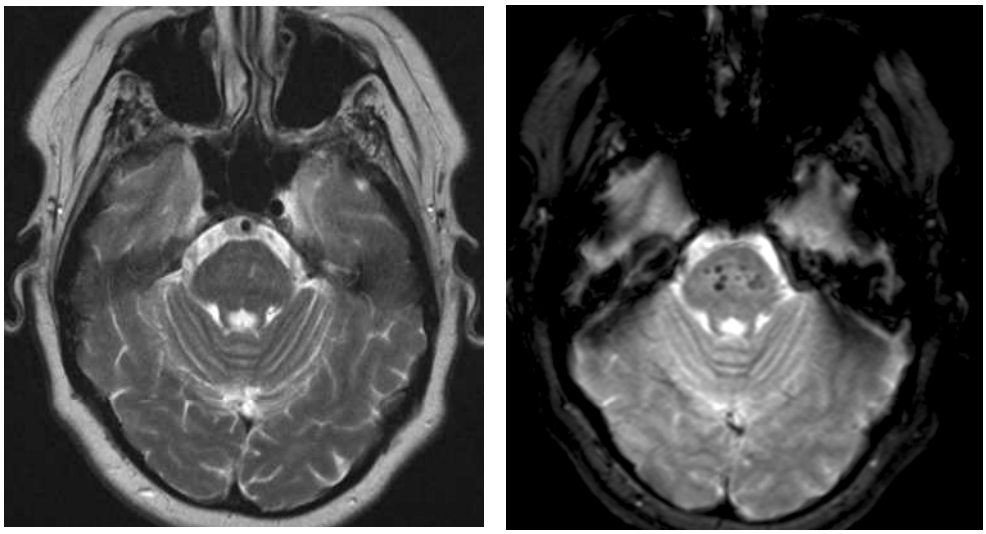


Figure 38. MRI example of a CMB (2).

T2-Weighted MRI (left panel) and GRE T2*-weighted MRI (right panel) from a patient with severe hypertension who was admitted with a clinical lacunar syndrome due to a small subcortical haemorrhage. She had stopped her antihypertensive medication before admission. Numerous CMBs are visible in the brainstem on GRE T2*-weighted MRI but not on T2-weighted MRI.

To date, there has been general agreement on a CMB cut-off size of 5-10 mm in diameter on standard GRE T2*-weighted MRI sequences, although in some studies, the minimum diameter used was 2 mm (Cordonnier et al., 2007). However, it should be appreciated that the measurement used for a radiological lesion is not a true reflection of the actual size of the CMB, due to the 'blooming' effect of the MRI signal at the lesion border (Greenberg et al., 2009a) meaning that the actual tissue lesion size is usually considerably smaller, and generally significantly less than approximately 5 mm (Schrag et al., 2010). Indeed, haemosiderin deposits exert a high susceptibility effect on the local magnetic field, which results in a larger area of signal loss (Greenberg et al., 2009b; Viswanathan and Chabriat, 2006). Some recent lines of evidence suggest that a rigid size definition for CMBs should not be emphasised. First, CMB size may be affected by imaging parameters including magnetic field strength and MR sequence characteristics, including the TE. Post-processing techniques including SWI can further increase the size, conspicuity and number of CMBs detected (Greenberg et al., 2009b). In fact, the smallest CMBs (< 1 mm) can be magnified up to threefold on SWI or high-resolution three-dimensional GRE T2* sequences because of the blooming effect (Greenberg et al., 2009b). Therefore, if an absolute size criterion is applied, the definition of CMBs even in the same subject could markedly change depending on which MRI sequence is used. Second, a recent study of lesion volumes in 46 patients with probable CAA showed that brain haemorrhage volumes in this population did not

form a single continuum between CMBs and macrobleeds. Rather, they showed a clearly bimodal distribution with a cut-off point of 5.7 mm, which interestingly falls within the lower end of the most commonly used CMB upper cut-off size of 5-10 mm (Greenberg et al., 2009b). Whether this finding holds true in other populations of patients with CMBs (e.g. those with IS) requires further investigation. For the moment, it remains reasonable to assume that radiological lesions much larger than approximately 5-10 mm should not be considered to be CMBs. Based on these principles, consensus MRI diagnostic criteria for CMBs have been proposed (Greenberg et al., 2009b) and are shown in Table 7. It should be noted though that these criteria do not include any indication of size for CMB identification.

Table 8. Recommended criteria for the identification of CMBs

From Greenberg et al., 2009b

Criterion	Rationale
1. Homogeneous hypointense (black) on T2*-weighted MRI or SWI	To ensure the lesion is paramagnetic and likely to contain blood degradation products
2. Well-defined rounded or ovoid lesions (rather than linear)	To exclude blood vessels and distinguish CMBs from cortical subarachnoid blood or superficial cortical siderosis (the latter may have separate relevance for diagnosing small vessel arteriopathies)
3. 'Blooming' effect on T2*-GRE MRI and SWI compared to T1- or T2-weighted sequences	Ensures that the lesion has susceptibility effect
4. Devoid of signal hyperintensity on T1-weighted or T2-weighted sequences	To avoid misclassifying some mimics, including cavernous malformations (bright on T2), metastatic melanoma (bright on T1)
5. At least half of lesion surrounded by brain parenchyma	To include very superficial cortical lesions which may be seen in CAA
6. Distinct from other mimics such as iron or calcium deposits, bone, or vessel flow voids	A reminder to consider these mimics
7. Clinical history excluding traumatic diffuse axonal injury	To avoid mixing secondary traumatic CMBs with 'primary' CMBs caused by small vessel disease

2.2.3 Cerebral microbleed mimics

The correct identification of CMB mimics is critical for the investigation of the prognostic and therapeutic significance of CMBs. CMB mimics have similar characteristics as CMBs on T1- and T2-weighted SE and GRE T2*-weighted MRI (Greenberg et al., 2009b). Common mimics to consider are mainly of two types: mimics that do not contain blood products (partial volume artefacts, paramagnetic substances: iron and calcifications) and mimics that do contain blood products (traumatic CMBs, cavernous malformations, haemorrhage with areas of infarction, flow voids from blood vessels, haemorrhagic metastases). Examples are shown below. For each type, awareness of the imaging hallmarks and location characteristics will reduce misinterpretation with true CMBs.

Mimics that do not contain blood products

Paramagnetic substances

Calcium, manganese and iron are paramagnetic substances that may appear as foci of low signal on GRE T2* sequences. The most frequent mimic in older adults is mineralisation in the basal ganglia, which may be associated with disorders of calcium metabolism, extrapyramidal syndromes or neuropsychiatric disorders (Casanova and Araque, 2003). CT

may be helpful to distinguish susceptibility effects of calcification or iron deposits from CMBs (Figure 39). In the posterior fossa, physiological iron deposits also occur in the dentate nuclei.

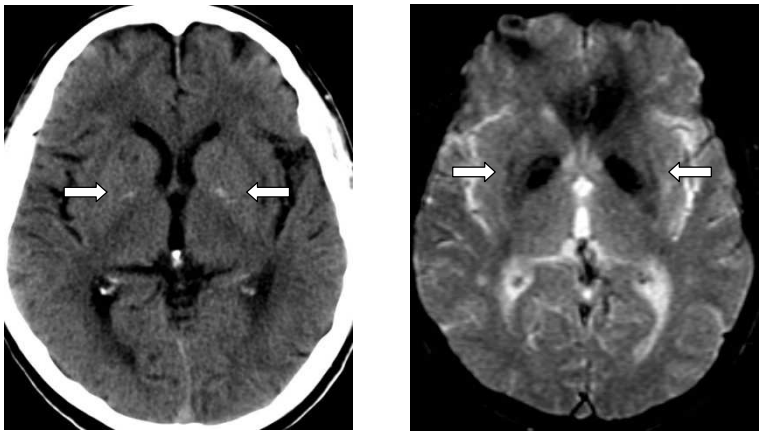


Figure 39. Basal ganglia mineralisation mimicking CMBs.
GRE T2* MRI demonstrates bilateral hypointense foci in the basal ganglia (right), corresponding to calcifications on axial CT brain (left).

Partial volume artefacts

In the brain, partial volume artefacts from adjacent bony structures can cause difficulties to the inexperienced observer. Partial volume artefacts result from the loss of signal to noise ratio secondary to the loss of contrast between different tissues. This may occur in different areas of the brain, i.e. the petrous temporal bones, paranasal sinuses, frontal bones, orbit and occipital bones (Figure 40). Problems from misclassifying CMB mimics can be minimised by careful inspection of adjacent slices and reference to T2, FLAIR and diffusion-weighted images.

Air embolism, iatrogenic devices and metallic embolism

A case of cerebral air emboli causing multiple, bilateral, small round foci of hypointensity on GRE imaging has been reported (Jeon and Kang, 2007). There are several descriptions of multifocal GRE hypointensities caused by embolization of metallic fragments from prosthetic heart valves (Almansori et al., 2008). Non-metallic ventricular shunt tubes in cross-section can also mimic CMBs (Tsushima and Endo, 2006).

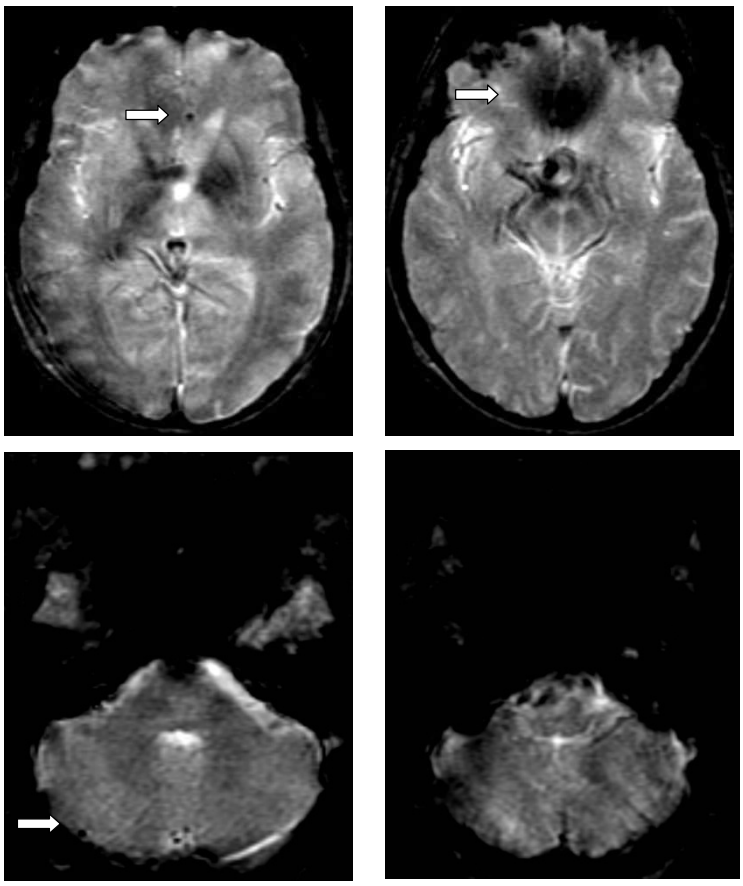


Figure 40. CMB mimic resulting from partial volume artefact on axial GRE T2* MRI

The partial volume artefact is adjacent to the frontal (upper panels) and occipital (lower panels) bones.

Mimics containing blood products

Blood vessels

Vascular flow voids in pial and leptomeningeal vessels in the cerebral sulci may be mistaken for CMBs on GRE T2* MRI, especially when seen in cross-section. Flow voids from vessels in the deeper structures of the brain can also be mistaken for CMBs. Keys to differentiate them from CMBs are the absence of any 'blooming' effect when compared with T2 (CMBs appear bigger on GRE T2 compared with T2-weighted MRI, whereas vessels do not), and their linear shape on consecutive axial slices (Figure 41).

Haemorrhagic transformation of an infarct

Identification of this particular type of mimic is possible from the clinical history and review of other MRI sequences (T2-weighted, FLAIR and DWI essentially). Small petechial haemorrhages may be seen within, or along the margin of, the infarct.

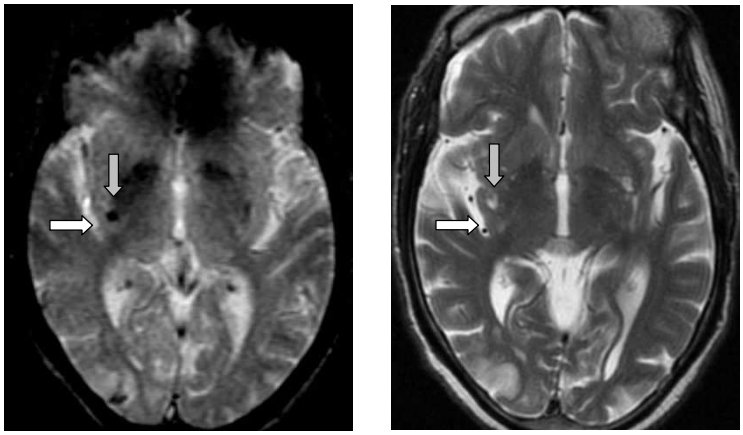


Figure 41. GRE T2* MRI (left) and T2-weighted MRI (right) of the brain showing the flow void of a leptomeningeal vessel.

The leptomeningeal vessel is imaged in cross-section (white arrow), and a petechial haemorrhage is visible within an area of infarction (grey arrow).

Cavernous malformations

Cavernous malformations are well-circumscribed nodules compsed of clustered dilated vessels without intervening neural tissue. They have been categorised into 4 types based on their MRI appearances (Raychaudhuri et al., 2005). Larger cavernomas have a characteristic hypointense rim of haemosiderin with a core of variable signal intensity giving them a characteristic 'popcorn' appearance (Rigamonti et al., 1987). Smaller (type IV) cavernomas appear as punctuate hypointense lesions on T2*-GRE images and can be indistinguishable from CMBs. To what extent these punctuate lesions, especially in isolation and without relevant family history or mutation, are distinct from CMBs is not clear.

Traumatic cerebral microbleeds

Traumatic brain injury causes rapid rotational acceleration and deceleration of the brain leading to shearing of axons in susceptible regions (e.g. gray-white matter junction, splenium of corpus callosum, internal capsule, dorsolateral brainstem) (Hortobagyi and Al-Sarraj, 2008). Traumatic CMBs can form as a result, but these CMBs are considered as CMB mimics as they are secondary to diffuse axonal injury rather than related to small vessel disease. The presence of other traumatic lesions e.g. skull fracture, contusions should alert the clinician of the correct diagnosis.

2.3 Prevalence

2.3.1 In healthy individuals

Both high risk populations and community based studies have shown a high prevalence of CMBs in the general population.

Overall prevalence

MRI studies using GRE sequences have demonstrated CMBs in 18% of individuals between the ages of 60 to 69 years and in 38% of individuals older than age 80 (Vernooij et al., 2008b). Earlier studies found CMBs in approximately 5-6% of the healthy elderly population (Roob et al., 1999b; Jeerakathil et al., 2004b; Cordonnier et al., 2007; Koennecke, 2006), and up to 11% in a community-based sample, a prevalence which further increased with age (Sveinbjornsdottir et al., 2008). Recent studies report a generally higher prevalence of CMBs than older studies, due to 2 factors: (1) advancements in imaging protocols with increased sensitivity and rating scales (Greenberg et al., 2009b) (2) testing of older cohorts, as with rising age, the incidence of hypertension, a strong risk factor for CMBs, increases (Roob et al., 1999b; Vernooij et al., 2008b).

The reported prevalence of CMBs in community-based cohorts varies by age and MRI methods used. Four population-based studies with reported prevalence on CMBs in normal individuals are reported in Table 8. These studies are: (1) the Austrian Stroke Prevention Study (ASPS); (2) the Framingham study; (3) the Age Gene/Environment Susceptibility (AGES)-Reykjavik study; and (4) the Rotterdam Scan study.

Overall, roughly 20% of the population shows evidence of small, focal areas of brain bleeding (Fisher, 2012), and by age 80, that prevalence is nearly 40% (Vernooij et al., 2008b). Even if these numbers are impressive, there may well be an underestimation of the actual prevalence of CMBs as the prevalence data are based on MR studies using a 1.5 Tesla field strength. If higher sensitive techniques (e.g., SWI, 3 Tesla MR) were used then these would show an even more robust prevalence (Nandigam et al., 2009).

Table 9. Prevalence of CMBs in normal individuals.

Cohort	No	Mean Age (years)	Prevalence of CMBs [No (%)]	MRI sequence	Inter-rater agreement (kappa)	Definition of CMB
Austrian Stroke Prevention Study	280	60	18 (6.4)	1.5 T, TR 600-800 ms, TE 16-20 ms, FA 20°, slice thickness 5mm, interslice gap 10%	0.4-0.65	2-5 mm
Framingham Study	472	64.4	22 (4.7)	1.0 T, TR 760 ms, TE 26 ms, FA 30°, slice thickness 5 mm, interslice gap 0.5 mm	0.33-0.57	<10 mm
AGES-Reykjavik study	1962	76	218 (11.1)	1.5 T, TR 3050 ms, TE 50 ms, FA 90°, slice thickness 3 mm, matrix 256 x 256, FOV 220 mm	0.71-0.73	No size limit
Rotterdam Scan Study	1062	69.6	250 (23.5)	1.5 T, high-resolution 3-dimensional T2*-weighted GRE sequence; TR 45 ms; TE 31 ms, FA 13°, slice thickness 1.6 mm, matrix 320 x 224	0.85-0.87	<10 mm

Differences of prevalence among different ethnic groups

In relation to cerebrovascular disease

CMBs are common in all types of stroke, but are more common in ICH than in IS (Cordonnier et al., 2007). Likewise, CMBs are more prevalent in patients with recurrent ischaemic and haemorrhagic stroke than in first-ever strokes (Cordonnier et al., 2007; Koennecke, 2006). Moreover, numerous studies have demonstrated that the frequency and extent of CMBs are associated with the severity of the white matter hyperintensities (Gorner et al., 2007; Jeong et al., 2004; Kato et al., 2002; Lee et al., 2005; Naka et al., 2004; Roob et al., 2000). This suggests that CMBs may be a biomarker of the severity or degree of progression of the underlying cerebrovascular disease. The development of CMBs is in close relation to the severity of cerebral microangiopathy. Histopathological investigations confirming this were detailed in section 1.3.1.

In a recent review of CMB studies, it was noted that in different stroke subpopulations, Asian samples tended to have a higher prevalence of CMBs than those based on presumably similar cases who were not of Asian ancestry (IS: 41.5% Asian versus 21.5% non-Asian; ICH: 68% Asian versus 47% non-Asian) (Cordonnier et al., 2007; Koennecke, 2006). A small pilot study suggested significant black-white racial differences in the

frequency and topography of CMBs in patients with ICH (Copenhaver et al., 2008), but it was limited by the small numbers and retrospective design. Although the analyses were adjusted for risk factors such as age and hypertension, other confounding factors may have played a role and were not adjusted for (e.g socio-economic status). Variations in prevalence among different ethnic and racial groups are difficult to interpret due to variations in the samples and methodologies, lack of adjustment for important confounding factors, and very scarce data.

In relation to Ischaemic stroke

The prevalence of CMBs in patients with IS varies widely, due to the heterogeneity of the stroke subpopulations studied and the etiological mechanisms in IS. Three studies have provided data on the detection rate of CMBs according to stroke subtype (Imaizumi et al., 2004; Kato et al., 2002; Naka et al., 2004). In spite of substantial heterogeneity in these three studies, the rate of CMBs in each subtype was as follows: 20% in atherothrombotic stroke, 23% in cardio-embolic stroke, and 53% in small vessel occlusion. Based on a pooled analysis of 1075 patients, the prevalence of CMBs among people with IS is 33.5% (range: 12 to 71%) (Cordonnier et al., 2007) and this prevalence increases with age (Imaizumi et al., 2008). In patients with IS, differences in prevalence of CMBs are also observed among different ethnic groups: CMBs are more prevalent among Asian subjects than non-Asian (Cordonnier et al., 2007).

Non-traumatic intracerebral haemorrhage

The overall prevalence of CMBs in non-traumatic ICH is 60.4%, ranging from 47% to 97%, which is considerably higher than in IS cohorts (Cordonnier et al., 2007). In patients with deep ICH, the prevalence of CMBs increases with age (Kwa et al., 1998). Differences in the prevalence of CMBs are also observed among different ethnic groups; CMBs are more prevalent among Asian subjects than non-Asian, possibly due to confounding by the higher prevalence of hypertension in Asian studies (Cordonnier et al., 2007) but in parallel with the higher proportion of ICH in stroke in Asian populations (Koennecke, 2006). When extrapolating data from black and white populations with ICH, blacks have a significantly higher incidence of ICH than whites, being 17.5/100.000 versus 13.5, probably because of the higher prevalence of hypertension among Blacks (Brott et al., 1986). However, in that study, blacks were younger (mean age 58 versus 67 for whites) and more often

hypertensive (74% versus 40%). The risk of ICH for hypertensive blacks was 4.4 times that for normotensive blacks (Brott et al., 1986).

2.3.2 Cerebral microbleeds in other groups and diseases

Patients with memory impairment

In a memory clinic setting the prevalence of CMBs was found to be higher (17%) than described in community samples, yet lower than in stroke patients (Cordonnier et al., 2006). Interestingly, their incidence varied significantly according to diagnostic groups and increased with increasing severity of cognitive impairment (10% of patients with subjective complaints, 20% with mild cognitive impairment (MCI), 65% with vascular disease) suggesting a possible link of CMBs with VCI. In AD, CMBs were reported in 26.8% of subjects (Hanyu et al., 2003a) and another study of vascular disease also found a high prevalence of CMBs in this population (Seo et al., 2007), thereby supporting this idea.

There is also some evidence that as the load of CMBs increases, brain function (e.g. cognition) may be affected (Goos et al., 2009; Werring et al., 2004), though whether CMBs directly and independently impact on brain function or are simply associated with the general severity of small vessel diseases is not yet known. There are few prognostic studies, but some data in a variety of different cohorts suggests that CMBs have a graded relationship with VCI (Staekenborg et al., 2009) and functional decline or death (Greenberg et al., 2004a; Henneman et al., 2009).

CADASIL

CMBs are also observed in some specific cerebrovascular conditions. Their overall prevalence in CADASIL is 37.5%, ranging from 25 to 69% (Lesnik Oberstein et al., 2001; Dichgans et al., 2002; Viswanathan and Chabriat, 2006; van den Boom et al., 2003). A large two-centre cohort study of patients with CADASIL recently defined risk factors for CMB which were detected in 35% of patients: the number of CMBs was independently associated with systolic BP (Odds ratio [OR] 1.42 risk per 10 mmHg increase, $p = 0.005$), the diagnosis of hypertension (OR 5.19, $p<0.001$), normalised white matter hyperintensities

lesion volume (OR 1.16 risk per percent increase in volume, $p < 0.001$) and total normalised lacunar infarct volume (OR 1.96, $p = 0.004$) (Viswanathan and Chabriat, 2006). These results suggest that vascular risk factors such as hypertension may promote microhaemorrhage from fragile small vessels prone to bleeding due to ultrastructural vessel wall modifications caused by the NOTCH 3 mutation.

Moyamoya

CMBs are also very frequent in moyamoya disease, and are found in up to 44% of Asians with this disease, depending on the type of MRI sequence used (Kikuta et al., 2005). CMBs tend to occur in a periventricular distribution, in the temporal subcortical region, and basal ganglia (Ishikawa et al., 2005). One study found that multiple CMBs may confer an increased risk of subsequent ICH (Kikuta et al., 2008). Interestingly, a recent report suggested that there is no association between CMBs and moyamoya-like vessels in patients with atherosclerotic steno-occlusive disease (Tanaka et al., 2012).

2.4 Risk factors

With the growing interest in CMBs over the past decade, clinical and radiological risk factors for CMBs are now reasonably well defined, the most important being increasing age, arterial hypertension, neuroimaging correlates of cerebral small vessel disease (white matter hyperintensities and lacunes), and APOE genotype (Koennecke, 2006; Werring, 2007). Ageing and chronic hypertension are the most consistent predictors of CMBs (Cordonnier et al., 2007; Fiehler, 2006; Henskens et al., 2008; Jeerakathil et al., 2004b; Koennecke, 2006; Kwa et al., 1998; Roob et al., 1999b; Tanaka et al., 1999; Vernooij et al., 2008b; Werring et al., 2005; Greenberg et al., 2009a; Sveinbjornsdottir et al., 2008). Other frequently reported risk factors include diabetes, cholesterol and antithrombotic drug use, which have been shown in some, but not all, studies to increase the likelihood of CMBs.

2.4.1 Demographic factors

Most studies with a reasonable sample size and age distribution show CMB prevalence increases with age (Cordonnier et al., 2006; Sveinbjornsdottir et al., 2008; Vernooij et al., 2008b). An association with the male sex and increased CMB prevalence was found in two studies (Jeerakathil et al., 2004b; Sveinbjornsdottir et al., 2008). Black-white and Asian-non Asian differences in the frequency and topography of CMBs have been noted in various studies, but such comparisons are difficult to interpret due to heterogeneity in samples.

2.4.2 Hypertension and other vascular risk factors

Hypertension is a recognised risk factor for CMBs. In patients with first-ever lacunar stroke, high BP defined according to measures of ambulatory day and night BP was independently associated with CMBs, with the association being the strongest for CMBs in the deep regions of the brain (Staals et al., 2009). In the PROSPER study, only age and hypertension distinguished elderly subjects with CMBs from those with no CMBs (van Es et al., 2008). Again in this study, the association was the strongest for CMBs in the deep structures. However, CMBs in the cortico-subcortical areas were also associated with an increased risk of hypertension. In light of this it was suggested that CMBs may be a manifestation of hypertensive-related microangiopathy that has reached an advanced stage in which blood vessels are prone to bleeding (Kato et al., 2002; Werring, 2007). However, the close correlation between ageing and hypertension itself may warrant further investigation into this matter (Koennecke, 2006).

Another way of looking at the association between CMBs and hypertension is to measure target organ damage, i.e. cardiac ventricular wall thickness , aortic stiffness, glomerular fitration rate (a measure of kidney function) or presence of retinopathy. Left ventricular wall thickness, a marker of hypertension severity and duration, is associated with an increased risk of CMBs (Lee et al., 2004b). In a study of patients referred for hypertension, the presence of aortic stiffness (determined by aortic pulse wave velocity and central BP) was not associated with CMBs but with imaging markers of CMBs (WML and lacunar stroke) (Henskens et al., 2008). The risk of CMBs increases 1.8 to 1.9 fold with each standard deviation (SD) rise in BP (Henskens et al., 2008). Likewise, a decrease in glomerular filtration rate was significantly associated with CMBs in a study of 152 patients with acute IS (Cho et al., 2009b).

There is increasing evidence that hypertension may influence the risk of developing new CMBs over time. Excellent control of hypertension is advocated to avoid the risk of developing CMBs; however, it has been seen that despite strict control of BP new CMBs appear on follow up MRI scans in hypertensive patients with ICH (Imaizumi et al., 2003). This topic will be developed further in Chapter 6.

CMBs are also associated with other vascular risk factors including smoking (Kwa et al., 1998), diabetes (Cordonnier et al., 2007), previous ICH (Greenberg et al., 2004a), low total serum cholesterol concentrations and low-density lipoprotein cholesterol (Lee et al., 2002). In the population-based Rotterdam Scan study (Vernooij et al., 2008b), there was a significantly increased likelihood that CMBs would be detected in users of any antithrombotic drugs, with a slightly stronger association in those using platelet aggregation inhibitors than in those using anticoagulant drugs. The risk associated with these drugs had similar magnitude to that for WML (OR 1.6 and OR 1.7, respectively), after controlling for age and sex. Risks were similar for strictly lobar and deep or infratentorial CMBs. The study further suggested that aspirin use may increase the likelihood of CMBs (Vernooij et al., 2009).

2.4.3 Pathological and radiological diagnoses underlying cerebral microbleeds

There is robust evidence that imaging and pathological markers of hypertensive arteriopathy and amyloid angiopathy increases the likelihood of CMBs. On pathology

specimens, these bleeding-prone microangiopathies are termed lipofibrohyalinosis when related to hypertension (Fazekas et al., 1999) and amyloid angiopathy when characterised by the deposition of amyloid protein in the cerebral small vessels (Good et al., 1998). CMBs have been associated with lacunes and extensive WML (leukoaraiosis) which have been reported in both hypertensive arteriopathy and CAA (Cordonnier et al., 2007; Fiehler, 2006; Greenberg et al., 2009b; Kato et al., 2002; Koennecke, 2006; Maia et al., 2006; Roob et al., 1999b; Tanaka et al., 1999; Werring, 2007; Werring et al., 2005).

CAA is a major cause of lobar ICH and cognitive dysfunction in the elderly and is related to a high prevalence of markers of small vessel disease, like WML and CMBs (Greenberg et al., 1996; Viswanathan et al., 2008). As such, patients with possible or probable CAA (for which the APOE ε4 allele is a marker) have been found to have significantly more often strict lobar CMBs compared to non-carriers (Sveinbjornsdottir et al., 2008; Vernooij et al., 2008b; Werring, 2007). Conversely, cardiovascular risk factors, like hypertension, the presence of lacunes and WML, are associated with CMBs in infratentorial (brainstem, cerebellum) or deep (basal ganglia, thalamus) locations; and hence, CMBs in these regions may be indicative of hypertensive/arteriosclerotic microangiopathy (Lee et al., 2004b; Roob et al., 1999b; Vernooij et al., 2008b). However, trying to divide the distribution according to CAA or hypertensive arteriopathy is likely to be an oversimplification as patients with CAA are often hypertensive and may present with a mixed distribution of CMBs.

2.4.4 Genetic risk factors for cerebral microbleeds

Most genetic diseases exhibiting an increased susceptibility to CMBs are rare. Mutations identified in CAA are characterised by misfolding of proteins, including Aβ, cystatin C, transthyretin and prions (Launer, 2011). CADASIL is a pure form of brain vasculopathy caused by mutations in the NOTCH 3 gene (Chabriat et al., 2009) and CMBs are detected in about a third of patients. Moyamoya is another example of genetic vasculopathy due to mutations in genes responsible for encoding proteins in smooth muscle cells (Guo et al., 2009), and is frequently associated with CMBs.

APOE is the only candidate susceptibility gene identified as risk for CMBs (Launer, 2011). Associations of the APOE ε2 and APOE ε4 alleles with both ICH (particularly lobar) and CAA have been described (Biffi et al., 2010a; Rannikmae et al., 2013; Sudlow et al., 2006; Tzourio et al., 2008). The mechanisms by which the APOE ε2 and APOE ε4 alleles cause CAA were developed in section 1.5. Strictly lobar CMBs are considered a diagnostic marker of

CAA; they have been included in the modified Boston criteria for CAA (Linn et al., 2010). There is now robust evidence that APOE ε4 carrier state is associated with CMBs (Cordonnier et al., 2007; Fiehler, 2006; Greenberg et al., 2009b; Kato et al., 2002; Koennecke, 2006; Maia et al., 2006; Roob et al., 1999b; Vernooij et al., 2008b; Werring, 2007; Werring et al., 2005; Maxwell et al., 2011).. A recent metanalysis of the genetic associations with CMBs suggested that APOE ε4 allele carriers are at higher risk of having CMBs, particularly in strictly lobar brain locations (Maxwell et al., 2011). The association between ε2+ genotypes and strictly lobar CMBs was not significant in the analyses. Given the reported associations of APOE genotype with CAA and lobar ICH (Sveinbjornsdottir et al., 2008; Vernooij et al., 2008b), this suggest that strictly lobar CMBs are a possible biomarker of CAA. It is likely that with advances in technologies, more single nucleotide polymorphisms will be identified.

CMBs have been reported in rare conditions caused by mutations that have been linked with small vessel disease. Mutations in the genes encoding for some components of the vascular basement membrane, such as type IV collagens, and forming the supporting structure of the membrane, have recently been identified as risk factors for small vessel disease in the brain. These mutations cause alterations to the vascular basement membrane and may predispose to small vessel disease and haemorrhagic stroke. Type IV collagens, and their most abundant types COL4A1 and COL4A2, are ubiquitous basement membrane proteins. A novel COL4A1 mutation has recently been reported in a normotensive patient with a history of infantile hemiparesis who later developed spontaneous, recurrent ICHs (Vahedi et al., 2007). A brain CT at age 23 showed diffuse leukoencephalopthy and multiple deep, hypodense lesions surrounded by calcifications suggestive of vascular cavities and MRI showed numerous CMBs.

Chapter 3 - Clinical Implications of Cerebral Microbleeds

The objectives of this chapter are to review the current knowledge on the impact on CMBs on clinical factors including cognition, cerebrovascular disease risk and on the safety of the use of antithrombotic and thrombolytic treatments. It will also discuss other clinical manifestations that have been associated with CMBs including transient attacks, and their link with disability and death. At the end of this chapter the aims of this thesis will be developed.

3.1 Cognitive impairment

In this first section it will be discussed whether CMBs independently influence cognitive functions. CMBs may be relevant in the study of cognition in healthy individuals, in patients with AD and cerebrovascular disease, and in the study of VCI. A summary of the studies on the impact of CMBs on cognition in different populations is given in Table 9 and these are discussed in more detail below.

3.1.1 Cerebral microbleeds in normal populations

CMBs have been detected in about 5% of healthy individuals with no known neurological disorder (Jeerakathil et al., 2004a; Roob et al., 1999b; Vernooij et al., 2008b). The prevalence increases with age (Hachinski et al., 2006) and with the type of imaging method used for CMB detection (Greenberg et al., 2009b). In one population-based study which used an optimised SWI sequence, the prevalence of CMBs was about 40% in those over 80 years old (Vernooij et al., 2008b). However, studies of how CMBs could affect cognitive function in healthy populations have been scarce. One recent study included 67% of adults attending a self-funded screening examination in Japan, and CMBs were found in 6.8% of these participants (Yakushiji et al., 2008), mainly in the cerebral lobes and deep white matter, with a predilection for the frontal lobes. Lower scores on the mini mental state examination (MMSE) were associated with the presence of CMBs, a shorter duration of education and the presence of severe white matter hyperintensities. In particular, 'attention and calculation' subscores were lower in those individuals with CMBs than those without. The authors speculated that CMBs may have direct effects of disrupting frontal-subcortical circuits to account for this pattern of cognitive impairment. One criticism of this study is that because participants were self-referring, the results may not be generalisable to other populations. In another hospital-based study of 772 patients attending a memory clinic, CMBs were detected in 10% of patients judged to have 'subjective' memory complaints, but the authors found no difference overall in cognitive function between patients with and without CMBs (Cordonnier et al., 2006). Of note, in this study, 54% of the patients with CMBs had only a single lesion, which might not be as likely to be associated with significant cognitive impairment as multiple lesions.

Table 10. Associations between CMBs and cognitive function in different populations*

*Adapted from Werring et al., 2010

Study reference	Design	Clinical population	Main associations	Other MRI markers adjusted for in the study
Yakushiji et al., 2008	Cross-sectional	Healthy self-funding adults (N=678)	Lower scores on MMSE associated with CMBs	WML
Cordonnier et al., 2006	Cross-sectional	Memory clinic (N=772)	None demonstrated	-
Werring et al., 2004	Cross-sectional	Stroke and TIA clinic (N=55)	Executive dysfunction more common in the group with CMBs compared with a control group	WML (ARWMC rating scale)
Seo et al., 2007	Cross-sectional	Memory clinic. Subgroup with subcortical vascular dementia (N=86)	CMBs associated with MMSE score and tests in a range of cognitive domains with exception of language	WML (Scheltens scale), lacunes
Hanyu et al., 2003	Cross-sectional	Memory clinic. Clinical diagnosis of AD (N=59)	No association with MMSE	-
Pettersen et al., 2008	Cross-sectional	Memory clinic. Clinical diagnosis of AD (N=80)	No association with MMSE or single test of a cognitive test battery	-
Goos et al., 2010	Cross-sectional	Memory clinic. Clinical diagnosis of AD	Multiple CMBs associated with lower MMSE (but few patients with multiple CMBs)	WML. No independent association found.
Viswanathan et al., 2008	Cross-sectional	CADASIL (N=147)	Association with global measures (MMSE, Mttis Dementia Rating Scale)	WML, lacunes. No independent association demonstrated except a small strategic effect for caudate/internal capsule CMBs
Liem et al., 2009	Longitudinal, 7 year results	CADASIL (N=25)	Increase in CMBs associated with deterioration on CAMCOG	WML, lacunes. No independent association reported.

3.1.2 Cerebral microbleeds in Alzheimer's disease

CMBs are commonly found in patients with AD, with a reported prevalence in hospital-based cohorts of clinically diagnosed AD of between about 20 to 30% (Cordonnier et al., 2006; Hanyu et al., 2003a; Pettersen et al., 2008). A mainly lobar distribution has been found, with in one study reporting that they were particularly affecting the occipital regions (Pettersen et al., 2008). This distribution is similar to that described in sporadic CAA, suggesting that CMBs in AD are more likely to be related to CAA rather than hypertensive arteriopathy. A recent MRI-post-mortem correlative study of the brains of eight patients with AD found that in microhaemorrhages where the source vessel could be identified, Aβ was present in the vessel wall, thereby supporting this hypothesis (Schrag et al., 2010). There are few studies investigating whether CMBs are associated with cognitive dysfunction in AD. One study of 80 cases of probable AD found no relationship between the presence of CMBs and cognitive impairment as assessed via tests of global cognition and focal domains (Pettersen et al., 2008). Two other studies found no relationship, but used the MMSE test, which may not be sensitive to subtle or focal cognitive deficits (Cordonnier et al., 2006; Hanyu et al., 2003a; Pettersen et al., 2008). A more recent study investigated 63 patients with AD, and compared cognitive test results in those with multiple compared to fewer CMBs (with a cut off set at 8 CMBs) (Goos et al., 2009). These results indicated that patients with multiple CMBs performed worse on the visual association test, digit spans and animal naming, when adjusted for other clinical and imaging confounding factors (Goos et al., 2009).

3.1.3 Cerebral microbleeds in cognitive impairment

MCI is the preclinical age-related cognitive impairment and is likely more prevalent than VCI. As part of the VCI entity, it represents a key healthcare challenge facing all aging Western societies and is second only to AD as a cause of dementia (Hachinski et al., 2006). Initial concepts of VCI invoked cortical or subcortical infarction – leading to the terms 'multi-infarct dementia' or 'post-stroke dementia' (Werring et al., 2010). However, subcortical small vessel disease (often not causing acute or overt clinical symptoms) also plays a critical role in VCI (Black et al., 2009; Bowler, 2007; van Dijk et al., 2008). MRI is the most important tool for detecting and quantifying small vessel disease, and forms part of the current diagnostic criteria for vascular disease (Hachinski et al., 2006). MRI manifestations of small vessel pathology including white matter hyperintensities, lacunes and perivascular spaces have been recognised for many years. Research on white matter

hyperintensities and cognition has shown only modest correlation, particularly in patients with symptomatic cerebrovascular disease (Sabri et al., 1999; van Swieten et al., 1996), which may be a reflection of both the pathological heterogeneity of small vessel disease, and the lack of pathological specificity of conventional MRI. The tissue water content forming the MRI signal may be affected by many pathological processes including infarction, ischaemic demyelination and gliosis, which may have different functional effects. There is thus a need for a better understanding of the pathological basis of MRI changes, and for more specific imaging markers to detect and quantify small vessel pathology. CMBs are now recognised as a manifestation of small vessel pathology (Greenberg et al., 2009b), but their clinical impact on cognition remains uncertain (Koennecke, 2006). Chapter 7 presents the work addressing the question of how CMBs might be related to cognition.

Cerebrovascular diseases

CMBs have been found in about 35% of patients with first-ever or recurrent IS, and about 60% of patients with first-ever or recurrent ICH (Cordonnier et al., 2007). A major challenge in any study to investigate the potential relationship between CMBs and cognitive function, particularly in stroke patient populations, is the strong association between CMBs and other radiological markers of cerebrovascular disease. One study undertaken in a neurovascular clinic population compared detailed cognitive function assessment in consecutive patients with CMBs (N=25) to a non-CMB control group (N=30) carefully matched for imaging and clinical factors likely to influence cognition, including white matter hyperintensities. A marked difference in the prevalence of executive dysfunction was found: 60% of patients with CMBs were impaired on tests sensitive to frontal 'executive' dysfunction, compared to 30% of patients without CMBs (Werring et al., 2004). Frontal 'executive' impairment was also related to CMB location in the frontal lobes or basal ganglia. This is consistent with the possible direct effect of CMBs on frontal-subcortical circuits, although the cohort studied was small. More recently, CMBs have been studied in cohorts of patients with a diagnosis of vascular disease. Seo et al. (2007) investigated 86 subjects with subcortical vascular disease from a memory clinic with GRE T2*-weighted MRI and neuropsychological testing, MMSE and a clinical dementia rating scale. The authors reported that CMBs were found in 85% of these patients; CMBs were related to the MMSE score, and to dysfunction in all cognitive domains except language, which was borderline significant. In a memory clinic population, Cordonnier et al. (2006)

found CMBs in 65% of patients with vascular disease, compared to 18% of patients with a diagnosis of AD. However, in this memory clinic population as a whole, CMBs were not related to cognition as assessed by the MMSE score. In CADASIL, a 'pure' form of cerebral small vessel disease, CMBs were associated with lower MMSE scores, but had no independent effects except for a possible strategic effect when located in the caudate nucleus (Viswanathan et al., 2007). These cross-sectional studies suggest that CMBs may play a role in VCI, either through direct structural or functional effects on surrounding tissues including white matter tracts, or as a marker for the general severity of small vessel damage.

Post-stroke dementia is an important subcategory of VCI, affecting about 10% of patients in the months following first-ever IS, and about 30% of patients after recurrent IS (Pendlebury and Rothwell, 2009). The possible mechanisms of dementia after stroke include either further episodes of infarction, or deterioration in small vessel disease not associated with clinical infarction. One hypothesis is that patients with pre-existing small vessel damage or AD are at increased risk of cognitive decline after an incident of clinical stroke (Hennerici, 2009). CMBs may therefore play a role as a prognostic marker of future cognitive decline or outcome in stroke patients. In support of such a hypothesis, data from a preliminary 5-year follow-up study of patients from a neurovascular clinic suggest that baseline CMBs may influence cognitive impairment at follow-up (Gregoire et al., 2009) (see Chapter 7). Another study in a memory clinic setting found that CMBs (particularly in association with brain atrophy) were an independent predictor of mortality (Henneman et al., 2009). Although further larger prospective studies are required, if reliable MRI markers of small vessel damage could identify a subgroup of patients at highest risk of subsequent cognitive impairment, these patients could be offered targeted control of their vascular risk factors, particularly hypertension (Birns et al., 2009; Hennerici, 2009).

Cerebral amyloid angiopathy

CAA is characterised by the vascular deposition of Aβ protein, mainly in cortical and leptomeningeal small calibre vessels, which provides a potential mechanism for vascular and cerebral dysfunction (Iadecola et al., 2009). CAA is generally recognised in life by symptomatic ICH, but at autopsy it is seen in up to 40% of the general elderly population, and in over 80% of brains affected by AD. There is evidence that severe CAA may be associated with cognitive impairment independently of lobar macrohaemorrhage (Greenberg et al., 2004b). Furthermore, population-based autopsy studies have shown that

CAA pathology is associated with cognitive impairment in life (Greenberg et al., 2004b). Because CAA is so common in the ageing brain, regardless of symptomatic ICH, it could be an important contributor to VCI. Population-based autopsy studies have shown that CAA is associated with more severe cognitive impairment in life. In addition to lobar ICH, CAA is increasingly suspected when lobar CMBs are detected on GRE T2*-weighted sequences. The presence of strictly lobar haemorrhages in patients over 60 years with a symptomatic lobar ICH has been reported to be highly correlated with severe CAA on histological analysis (Knudsen et al., 2001), though it is unclear whether CMBs in deep regions should be an absolute criterion. Moreover, the specificity of the Boston criteria for CAA in clinical practice is not fully established, since the validation studies included small selected groups of patients who did not undergo standardised imaging protocols (e.g. not routinely using T2*-weighted MRI). Further studies are therefore needed to determine how generalisable the Boston criteria are to other cohorts of patients with ICH. If CMBs are related to cognitive dysfunction, then the question arises as to whether this is a direct effect on surrounding tissues, an indirect effect (e.g. arteriolar narrowing causing hypoperfusion and ischaemic damage), or simply a marker for severe small vessel pathology. One recent study investigated pre-ICH cognitive impairment in 49 individuals with clinically probable CAA, and found that the mean apparent diffusion coefficient (ADC) in the brain was strongly correlated with cognition, whilst other MRI markers (white matter hyperintensities, number of CMBs, atrophy measures) were not (Viswanathan et al., 2008). These data suggest that in CAA, CMBs may exert any functional effects on cognition indirectly, perhaps by chronic hypoperfusion or neuronal degeneration related to focal lesions.

3.2 Cerebrovascular disease risk

3.2.1 Ischaemic stroke

The deep perforating arteries feeding the areas of the brain harbouring CMBs show moderate to severe lipohyalinosis and ruptured arteriosclerotic microvessels secondary to chronic hypertension (see Chapter 2). These changes form the substrate of the association between CMBs with ischaemic vascular diseases which has been demonstrated in many studies to date (Cordonnier et al., 2007). The prevalence of CMBs in patients with IS varies widely suggestive of heterogeneity of pathology (mechanisms of stroke) and differences in cohort studied (Lee and Roh, 2011). The prevalence of CMBs in patients with previous TIA, cerebral infarction or recurrent IS is higher than that in healthy adults (Cordonnier et al., 2007; Naka et al., 2004; Werring et al., 2004). Depending on studies, the overall prevalence in first IS is about 20-30% and up to 44% in recurrent IS, suggesting that CMBs are associated with the progression of small vessel cerebrovascular disease (Cordonnier et al., 2007). CMBs seem to predominate in patients with small vessel occlusion-type IS compared with patients with atherothrombotic and cardio-embolic strokes (Kato et al., 2002; Naka et al., 2004; Ovbiagele et al., 2006).

Whether CMBs are a marker of increased future IS risk is a question of potentially major clinical significance. An accurate estimation of risk is essential for the safe implementation of treatment and prevention strategies such as antithrombotic agents in patients with CMBs. Small prospective studies provide a partial answer to this question (Boulanger et al., 2006; Thijs et al., 2010). In a study of 487 European patients with IS or TIA, patients with CMBs had a higher risk of developing new IS but not ICH (Thijs et al., 2010). Recurrent ISs occurred in 10% of patients with CMBs and 5% of patients without CMBs; this difference almost reached significance ($p=0.054$). However, the association between CMBs and recurrent stroke was not independent (Thijs et al., 2010). In line with these results, Boulanger et al found an elevated risk of fatal and disabling cerebrovascular events, mainly ischaemic, in patients with CMBs in a small prospective study of Canadian patients with IS or TIA followed up over 14 months (Boulanger et al., 2006). There are a number of other small prospective studies in Asian populations which report the associations between CMBs and recurrent strokes (see section 3.3), but heterogeneity of the populations, differences in outcome measures (haemorrhagic and ISs) and methodologies make their findings difficult to compare. In most of these studies the exposure to antithrombotics was treated as a

dichotomous variable, which gives less accuracy in assessment of risk than duration and severity of exposure (e.g. type of antithrombotic) and represent another potential limitation. Like in risk assessment for ICH (see section 3.2.2), results are not generalizable across studies due to heterogeneity and differing ethnic backgrounds of populations studied. In a recent meta-analysis of 10 prospective cohorts involving 3067 patients with IS or TIA, there was a significant association between the presence of CMBs at baseline and the risk of recurrent IS in Western, but not Asian cohorts (Charidimou et al., 2013a). The presence of CMBs more than doubled the risk of recurrent IS (OR 2.23, 95%CI 1.29-3.85).

3.2.2 Intracerebral haemorrhage

CMBs have been associated with ICH in numerous studies. The overall prevalence of CMBs in patients with ICH ranges from 47% to 97%, a higher prevalence than in IS (Lee et al., 2005; Naka et al., 2004). Several cross-sectional and prospective studies indicated that the presence and burden of CMBs is strongly related to the incidence or recurrence of spontaneous ICH (Fan et al., 2003; Greenberg et al., 2004b; Lee et al., 2004c). They have been associated with the severity of ICH (Lee et al., 2006). They have also been considered to be pathogenetically related with ICH (see Chapter 2), and a regional association between CMBs and macroscopic ICH was demonstrated in several studies, suggesting similar underlying pathogenic mechanisms (Chen et al., 2008; Lee et al., 2004a). The spatial distribution of CMBs in ICH may be of clinical interest in understanding the causes of macroscopic ICH in vivo. Lobar CMBs are particularly frequent in patients with CAA, a predominant cause of ICH in the elderly (Greenberg et al., 1996; van den Boom et al., 2005). Analyses comparing hypertensive ICH and CAA-related ICH indicated that cortico-subcortical CMBs are more frequent in the CAA group (Lee et al., 2007). A distinct genotype for APOE was found in patients with lobar and non-lobar CMBs in a study of 414 consecutive stroke patients, suggesting a different pathogenesis between lobar and deep CMBs (Kim et al., 2005). This support the hypothesis that, in ICH, lobar and deep CMBs are attributable to different pathogenic mechanisms, CAA for lobar CMBs and hypertension for deep CMBs, although a clear-cut distinction is unlikely due to overlap of risk factors for the two processes in the elderly (e.g. hypertension).

As a marker of a bleeding-prone state in the brain, a major clinical question is whether CMBs are associated with an increased risk of subsequent haemorrhagic stroke particularly in relation to antithrombotic agents. In spite of the increasing interest and number of published studies attempting to address this question, their significance as a risk factor for

ICH remains unclear due to inconsistent study results. Studies showing an increased risk of ICH in patients with CMBs after IS have mainly been performed in Asian populations (Fan et al., 2003; Imaizumi et al., 2004; Naka et al., 2006; Soo et al., 2008) and might not be generalizable to other study populations. In patients with ICH, Jeon and co-authors found an elevated risk of haemorrhagic stroke due to CMBs in a prospective study of 112 survivors of ICH but the analyses could not be corrected for confounding factors due to small sample size (Jeon et al., 2007). Similarly, the haemorrhagic burden at baseline (either macro- or microhaemorrhages) predicted the appearance of new ICHs on repeat MRI after a mean interval of 16 months in a prospective study of 94 survivors of spontaneous ICH (Greenberg et al., 2004b). In patients with ICH and advanced WML, the presence and burden of CMBs was higher than in patients with similar degree of WML but no ICH, with a high positive predictive value of CMBs for ICH (Lee et al., 2005). The risk of recurrent bleeding after symptomatic ICH seems to be higher for lobar ICH (often presumed due to CAA) (Passero et al., 1995; Vinters, 1987) suggesting that lobar CMBs may be a stronger risk factor for antithrombotic-associated ICH than deep CMBs but data are lacking. A recent study of 104 survivors of spontaneous ICH attributed to CAA demonstrated that recurrent ICH was associated with baseline lobar CMBs (Biffi et al., 2010b). A recent meta-analysis demonstrated that CMBs are associated with a significant increased risk of ICH, especially in Asian populations (OR 10.43, 95%CI 4.59-23.72) (Charidimou et al., 2013a). There was a non significant trend towards a similar association in Western cohorts. However, the study was limited by small sample sizes, short follow-ups, few outcome events and selection bias. An overview of CMBs as risk lesions for ICH in the context of antithrombotic use will be discussed in the next section.

3.3 Antithrombotic treatment

ICH is one of the most important complications of long-term antiplatelet therapy (He et al., 1998) and of anticoagulants (Hart et al., 1995), and carries the worst prognosis of all acute cerebrovascular diseases (Broderick et al., 2007; Rosand et al., 2004). Identifying the risk factors for antiplatelet- and warfarin-induced ICH is crucial to improving the risk-to-benefit ratio of these drugs. CMBs, as a marker of subclinical cerebral damage due to small vessel disease, may predict the future risk of ICH in patients with ICH or IS (Fan et al., 2003; Greenberg et al., 2004a; Jeon et al., 2007; Naka et al., 2006; Soo et al., 2008). Studies have assessed the association between ICH risk and CMBs and the potential influence of the use of antithrombotic agents on the association. Antithrombotic drugs are often prescribed to elderly patients for IS prevention but may place them at risk of CMBs and more major bleeds. In addition to age, these patients often carry several risks factors for ICH and CMBs including hypertension, AD, CAA and diffuse cerebrovascular disease. In such patients, mortality from ICH may outweigh the benefits of antithrombotic agents. Anti-thrombotic agents only offer a modest absolute risk reduction of recurrent IS estimated at 0.69-2.49% per year for aspirin and 6% per year for warfarin (Antithrombotic Trialists Collaboration, 2002; Saxena and Koudstaal, 2004). Therefore, the benefit of anti-thrombotic agents has to be weighed with the risk of ICH, especially in the presence of CMBs. In the Birmingham Atrial Fibrillation Treatment of the Aged Study (BAFTA), patients with a mean age of 81.5 years followed up for a mean of 2.7 years and who received warfarin with a target international normalisation ratio (INR) of 2 to 3 had a 0.5% risk per year of ICH (Mant et al., 2007). The risks of major intracerebral bleeding associated with antithrombotic agents might be reduced if patients are carefully selected.

3.3.1 Antiplatelet use

Recent data suggests that the use of antiplatelet agents may be associated with a higher prevalence of CMBs in the healthy elderly population. In the Rotterdam Scan Study, which is a population-based, cross-sectional MRI study in the general elderly community in the Netherlands, the use of platelet aggregation inhibitors was significantly associated with the presence of CMBs; the association persisted after adjustment for cardiovascular risk factors and after excluding patients with a known history of cerebrovascular disease (Vernooij et al., 2009). However, a recent study of 1452 asymptomatic elderly subjects aged 65 years and above without cerebrovascular disease seems to challenge these results; in adjusted

analyses, there was no association between aspirin and the presence of CMBs even after prolonged aspirin use (Kim et al., 2012).

In patients with cerebrovascular disease, there has been some evidence that aspirin is associated with CMBs (Ge et al., 2011; Lovelock et al., 2010; Vernooij et al., 2008b). In a systematic review of antithrombotic use among European and Asian subjects, an excess of CMBs in antiplatelet users versus non users with ICH (OR 1.7) was documented (Lovelock et al., 2010). In patients with cerebrovascular disease stratified by specific antiplatelet agents, antiplatelet use more than doubled the risk of having CMBs in patients with ICH but not in patients with IS; the presence of CMBs was associated with the use of aspirin but not of other antiplatelet agents (Naka et al., 2013). However, results of these analyses need to be taken with caution. The systematic review mentioned is a case-case comparison study pooling observational data from very heterogeneous populations. It is possible that the role played by antiplatelet agents in such bleeds may have been overestimated, given variations in risk factor load between populations and variations in the level of control of important risk factors such as blood pressure. Furthermore the study was unable to establish causality due to its cross-sectional design. Nevertheless, other reports have found an association between antiplatelet use and CMBs (Jeong et al., 2004; Wong et al., 2003). Wong et al. (2003) found CMBs in 19 out of 21 aspirin users with symptomatic ICH, compared to 7 out of 21 matched aspirin users without ICH, but the study was limited by several factors: the absence of a group of patients with ICH unrelated to aspirin and the lack of adjustment for the presence of leukoaraiosis, a potentially important confounding factor. Another study found that in patients with ICH, the prevalence of CMBs was higher among users of antithrombotic agents, mainly antiplatelet medications (Jeong et al., 2004). Similarly, in patients with ischaemic cerebrovascular disease only (i.e. IS or TIA), CMBs were more frequent in aspirin users compared with non-users especially after long exposure to aspirin (greater than 5 years) although a confounding effect of cerebrovascular disease could not be completely eliminated (Ge et al., 2011). Likewise, Lovelock and co-authors found that antiplatelet use increased the risk of CMBs in patients with IS or TIA, but to a lesser extent than in patients with ICH (OR only of 1.4) (Lovelock et al., 2010). This confirmed the results of an earlier study in patients with cerebral infarction showing that previous use of antithrombotic drugs was independently associated with the presence of CMBs (Nighoghossian et al., 2002).

A major concern to physicians is whether patients with CMBs are more prone to ICH and hence may affect their treatment choice when antiplatelet drugs are required in secondary prevention of ischaemic cerebrovascular diseases. Several studies suggested that CMBs may increase the risk of antiplatelet-related ICH (Lovelock et al., 2010; Soo et al., 2008). Among 908 consecutive patients with acute IS admitted to a regional hospital in Hong Kong and treated with a single antithrombotic agent (mainly aspirin), Soo et al. showed that both age and CMBs were independent predictors of subsequent ICH (Soo et al., 2008). They further demonstrated that, in patients with 5 or more CMBs and treated with aspirin, the risk of ICH reached almost 8% and resulted in 4% mortality. As such, the risk of mortality from ICH may outweigh the benefit of antiplatelet agents, although the analyses could not be adjusted for severity of the white matter disease, another important contributor to ICH risk. A recent randomised trial comparing the new antiplatelet drug cilostazol to aspirin found that in the six patients who developed ICH, all had previous CMBs in the location of the ICH (Huang et al., 2008). These observations are consistent with the hypothesis that CMBs could be used to identify patients at the highest risk of antiplatelet-associated ICH, but the small sample sizes and small number of outcome events makes it difficult to draw firm conclusions with the available data.

The mechanisms implicated in the effects of aspirin exposure in patients with ICH and CAA is unclear. As suggested in the Rotterdam Scan Study, exposure to aspirin may be associated with increased prevalence of lobar CMBs, one of the hallmarks of CAA, and consequently increase the risk of recurrent ICH by substantially increasing the number of CMBs at risk for conversion into clinically manifest macrobleeds (Biffi et al., 2010b; Vernooij et al., 2009).

How does the current knowledge translate into medical practice? Published data does not provide sufficient evidence to withhold aspirin and platelet aggregation inhibitors in patients with CMBs. In the healthy elderly population, although it may be associated with a higher prevalence of CMBs, it is most likely to be safe. In the secondary prevention of thrombotic events, antiplatelet use is justifiable and most likely beneficial assuming future risk of ICH is low or can be reduced by controlling associated risk factors e.g. hypertension. In patients with ICH, despite a strong association between CMBs and ICH, recent data suggests that antiplatelet agent administration in patients with CMBs may not be as harmful as previously considered. Certain groups of ICH survivors at high risk for ischaemic cerebrovascular disease may benefit from antiplatelet agents without substantial increase

of risk of ICH recurrence. In a prospective cohort study, the regular use of antiplatelets for ICH patients was not associated with a substantial increase in risk of ICH recurrence (Viswanathan et al., 2006). Although lobar ICH location has consistently been associated with an increased risk of ICH recurrence (Bae et al., 1999; Douglas and Haerer, 1982), survival rates were similar between survivors of either lobar or of deep haemorrhages (Viswanathan et al., 2006). Similarly, in a prospective cohort study of 104 lobar ICH survivors with CAA, the use of aspirin after ICH was associated with lobar ICH recurrence when analyses were controlled for baseline predictors of ICH and CMB burden but the study did not have sufficient statistical power to confirm this (Biffi et al., 2010b). Moreover, in patients with ICH, pre-treatment with antiplatelet agents seems not to be an independent risk factor of mortality and unfavourable outcome (Foerch et al., 2006). These findings are consistent with the results of two large meta-analyses pooling the results of trials in subjects without a history of ICH which found that aspirin conferred only a small risk of intracranial bleeding (Antithrombotic Trialists Collaboration, 2002; He et al., 1998). Interpretation of such studies is difficult due to the fact that exposure to antiplatelet drugs is not randomly assigned but results from physician decisions based on individual patients. Also confounding by indication for antiplatelet use may have occurred (Viswanathan et al., 2006). For example, antiplatelets may have been avoided in patients at higher risk for ICH recurrence (e.g. history of previous ICH, lobar location of ICH, number of ICHs on scan) or in patients with higher numbers of lobar CMBs, leading to an underestimation of antiplatelets ICH-associated risk. Another problem relates to the lack of blinding of the readers of the MRI scans to the presence of ICH causing bias in reporting the number of CMBs. An ideal study design will need to prospectively collect information on antiplatelet use in different population categories comparing large groups of non-antiplatelet users and antiplatelet users in which antiplatelet therapy will be randomly assigned, specifying the indication for antiplatelet use, the duration of exposure to which particular agent, and recording the rates of new ICHs over several years of follow-up. Adjustments for all potential confounders including coexistent cerebrovascular disease and risk factors will be required as well as total blinding of the readers of the MRI scans.

3.3.2 Anticoagulant use

Anticoagulant use has risen sharply over the past decade due to increased frequency of AF in relation to aging of the population (Lip et al., 2012; Miyasaka et al., 2006). This has caused an increase in incidence of ICH in the elderly population (Flaherty et al., 2007;

Lovelock et al., 2010). Although highly effective in secondary prevention of ISs secondary to AF (Anon, 1994), warfarin use is associated with a significant increase in the annual risk of ICH, with devastating consequences including high disability and mortality (van Walraven et al., 2002). Because oral anticoagulation-induced ICH is so often fatal or disabling, even a small increase in the absolute risk of ICH can potentially outweigh the benefit of oral anticoagulant treatment (Charidimou et al., 2012c; Gustafsson et al., 1992). Thus, the prevention of ICH in patients on warfarin is critical and identification of risk factors remains an important issue.

As suggested by the fact that many ICHs occur while anticoagulation intensity is within the therapeutic range (Rosand et al., 2004), anticoagulant medications do not cause ICH on their own. Anticoagulant-induced ICH is likely to result from an interplay of different factors including the presence of an age-related underlying microangiopathy (mainly hypertensive-related or CAA), vascular risk factors, genetic factors and the use of oral anticoagulation treatments (Charidimou et al., 2012c). The Stroke Prevention in Reversible Ischaemia Trial (SPIRIT) assessed independent predictors of haemorrhage in 651 anticoagulated patients after cerebral ischaemia and pointed out that old age (>65 years), leukoaraiosis detected by CT and intensity of anticoagulation predicted warfarin-related ICH (Anon, 1996; Gorter, 1999). The increased risk of warfarin-related ICH associated with leukoaraiosis on CT was independent of age, BP and INR value (Gorter, 1999). In a case-control study of 26 patients with warfarin-related ICH following IS and 56 controls, Smith et al. confirmed that the presence and severity of leukoaraiosis detected by CT was an independent, dose-dependent risk factor for post-stroke warfarin-induced ICH (Smith et al., 2002). However, leukoaraiosis is a radiological definition which encompasses several disease mechanisms not necessarily all related to previous ischaemia (Gouw et al., 2011; Schmidt et al., 2011) and its predictive value for anticoagulation-related ICH is likely to be modest (Pantoni, 2010). Nevertheless, ischaemic leukoaraiosis is one of the two age-related damaging processes of the small brain vessels that contribute to ICHs in the elderly, possibly exacerbated by the use of anticoagulant treatments. We have indirect evidence that CMBs may increase the risk of warfarin-induced ICH by the close association of CMBs with leukoaraiosis (Hanyu et al., 2003b; Kato et al., 2002; Kwa et al., 1998; Naka et al., 2006; Naka et al., 2004). As CMBs are a marker of more advanced cerebrovascular disease and severe underlying microangiopathy, and presence of leukoaraiosis is a risk factor for warfarin-related ICH (Smith et al., 2002), CMBs might contribute to increase the risk of warfarin-related ICH.

CAA accounts for another large proportion of ICHs in the elderly, especially lobar (Charidimou et al., 2012b). It is likely implicated in the majority of the new lobar ICHs that have shown a sharp increase in incidence over the past 2 decades among patients aged 75 years and older (Lovelock et al., 2007) and may also account for the increased incidence of anticoagulation-associated ICH (Charidimou et al., 2012c). CAA was demonstrated in 7 of 11 lobar ICHs occurring on warfarin in a genetic and pathologic study (Rosand et al., 2000). Genetic risk factors might also contribute to warfarin-related ICH risk. Carrying the apoE ε2 or ε4 allele is associated with increased risk for lobar ICH and recurrent ICH resulting from CAA (McCarron and Nicoll, 2000; O'Donnell et al., 2000). Genetic polymorphisms may predispose to increased risk for high INR levels on warfarin resulting in lower dose requirements (Higashi et al., 2002).

CMBs detected on blood-sensitive MRI sequences, correspond to small collections of blood-breakdown products next to diseased vessels affected by hypertensive-related lipohyalinosis or CAA (see Chapter 2), and hence provide evidence of blood leakage from these vessels (Charidimou and Werring, 2011). As a result of minor bleeding from advanced microangiopathy, CMBs might serve as the substrate for large haemorrhagic strokes on warfarin. The presence of warfarin could result in a tiny bleed enlarging even under normal haemostatic mechanisms and would unmask an ICH that would have otherwise remained self-limiting and asymptomatic (Hart et al., 1995). CMBs have been associated with ICH in patients taking anticoagulant medications in cross-sectional case-control, case-case comparisons and small case series studies (Lee et al., 2009; Lovelock et al., 2010; Ueno et al., 2008) suggesting that they may be a stronger predictor of anticoagulant-associated ICH than leukoaraiosis (Charidimou et al., 2012c; Ueno et al., 2008). In a small case-control series comparing 24 patients with warfarin-related ICH versus 48 warfarin users who did not develop an ICH, Lee and co-authors reported that the presence of CMBs increased the risk of warfarin-associated ICH by 80-fold (Lee et al., 2009). In a systematic review of antithrombotic use in European and Asian cohorts, the frequency of CMBs in ICH patients treated with anticoagulants was reported to be 2.7-fold that in patients not treated with anticoagulants (with 'spontaneous' ICH) (Lovelock et al., 2010). Furthermore, Lee and co-authors suggested in a case-report that CMBs increase the risk of warfarin-related ICH based on the development of large haemorrhages at the site of previously identified CMBs (Lee et al., 2008a). However, these studies were small and unable to establish a causative relationship between CMBs and warfarin-related ICH risk. Limited prospective studies are available but suffer from serious methodological limitations mainly related to small sample

size and lack of standardised imaging criteria (Fan et al., 2003; Soo et al., 2008). In the largest prospective study to provide long-term clinical outcome in IS patients with CMBs treated with antithrombotic agents, Soo and co-authors showed that age and presence of CMBs were independent predictors of subsequent ICH (Soo et al., 2008). The risk of subsequent ICH increased with the number of CMBs from 0.6% (no CMB) to 7.6% (≥5 CMB) and mortality resulting from ICH in patients with ≥5 CMB was 3.8%. The high risk and mortality of ICH seemed to outweigh the modest benefit of antithrombotic agents (3% absolute risk reduction per year for aspirin, 6% absolute risk reduction for warfarin), suggesting that antithrombotic agents may cause more harm than benefit in patients with multiple CMBs. However, only 4% of patients in this study were treated with warfarin and results are mainly driven by aspirin. In another Asian cohort study, Fan and co-authors followed up 121 patients with IS over a mean of 27 months (Fan et al., 2003). Four patients with CMBs (9.3%) and one patient without (1.3%) had ICH (p=0.05). Again, only a small proportion of patients were using warfarin while the large majority were antiplatelet users and these findings might not be generalisable to other populations.

Whether duration or intensity of anticoaglution plays a role in the development of anticoagulation-induced ICH in patients with CMBs remains unclear. Duration of anticoagulation and presence of CMBs were similar in 141 patients with IS on warfarin and 105 controls with IS not on warfarin, suggesting that warfarin treatment may not contribute to CMBs and that other factors may play a role, e.g. a high prothrombin time (Orken et al., 2009).

Based on the available data, there is no reason to suggest that the risk of warfarin-related ICH is high enough to outweigh its benefit and that warfarin should be withheld in patients with CMBs (Soo and Wong, 2011). However, one will be extremely cautious about initiating anticoagulation therapy in patients with probable CAA as they are particularly prone to warfarin-related ICH and ICH recurrence (O'Donnell et al., 2000; Rosand et al., 2004). Anticoagulant therapy will be best avoided in these patients. Further data are required to determine the risk of anticoagulants in patients with strictly lobar CMBs. Although studies suggest a higher risk of anticoagulant-related ICH in these patients, CAA remains a pathological diagnosis and its diagnosis cannot be solely made based on the presence of strictly lobar CMBs. Therefore, care should be taken in controlling these patients' risk factors for ICH (e.g. strict BP control, close monitoring of INR) if anticoagulants are to be initiated. One should hope that recent, large prospective studies will clarify the risks of ICH

associated with CMBs. The Clinical Relevance for Microbleeds in Stroke Study-2 (Cromis-2) is a prospective, multicentre UK cohort study of patients anticoagulated after cardioembolic stroke, aiming at understanding the contribution of CMBs in the occurrence of ICH (www.ucl.ac.uk/cromis-2/).

To conclude this section, the association between CMBs and the use of antithrombotic drugs remains controversial. The available evidence relies on retrospective, case-control, observational or small prospective studies with insufficient power to reach definite conclusions. Main limitations of available studies were the short follow-up periods and insufficient number of outcome events. Other limitations are that most studies were conducted in Asian cohorts in whom hypertension and CMBs are more common than in Europeans and therefore may not be generalisable to other populations. Other limitations arise from the fact that, when studying CMBs, it is difficult to exclude that CMBs existed before the onset of use of these antithrombotic agents. This emphasises the need for prospective data to study the association between antiplatelet agents and anticoagulants in relation to CMBs and symptomatic ICH. In addition to safety of antithrombotic drugs, one will also be interested to know whether antithrombotic agents increase the rate of accumulation of CMBs over time and whether CMB distribution (i.e. lobar CMBs) plays a role in antithrombotic-related ICH risk. Information from GRE imaging will be valuable when deciding whether to prescribe antithrombotic medications, especially anticoagulants, after IS. Before formal guidelines emerge, precautions should be taken to minimise the risks of ICH in patients with CMBs, especially with a high load of CMBs. These include a stringent BP control, maintaining cholesterol levels within the normal range, close INR monitoring for patients on anticoagulants, and targeting a low INR window (2.0 to 2.5) in patients with AF and without prosthetic heart valves.

It was recently highlighted that because anticoagulant-related ICH remains a rare adverse event, randomised controls trials to investigate the safety and efficacy of anticoagulant drugs might not be the most appropriate method (Charidimou et al., 2012c). Selection bias related to patient's bleeding risk profile and adherence are likely to occur causing an underestimation of the bleeding risk. This is likely to hold true for antiplatelet-related ICH too which has a lower incidence than anticoagulant-related ICH. In such assessments of risk, correct estimates may be better provided by observational cohort studies (Charidimou et al., 2012c; Vandenbroucke, 2011). Here in Chapter 5, we present the results of a case-

control hospital-based cohort study of the risk of antiplatelet-related ICH in patients with CMBs.

3.4 Thrombolysis

Several studies have addressed the role of CMBs in the prediction of ICH or haemorrhagic transformation after thrombolysis in IS. Two initial observational studies indicated that presence of baseline CMBs predicted incident haemorrhagic transformation in acute IS patients after intra-arterial thrombolysis (Kidwell et al., 2009) and without thrombolysis (Nighoghossian et al., 2002). However, subsequent prospective multicentre studies failed to confirm the initial observation in patients receiving IV thrombolysis (Derex et al., 2004; Fiehler et al., 2007; Kakuda et al., 2005). In the BRASIL study, MRIs from 560 patients with acute IS recruited from 13 centres worldwide were analysed prior to thrombolysis. There was no significant increase in the rate of symptomatic ICH among the 86 patients with CMBs (Fiehler et al., 2007). The risk of symptomatic ICH among those with and without CMBs was compared with benefit of thrombolytic therapy from the literature. The authors concluded that risk of asymptomatic ICH would unlikely exceed the benefit of thrombolytic therapy but the study lacked statistical power to conduct subanalyses on subgroups at potentially higher risk (e.g. patients with numerous CMBs). Likewise, CMBs were not associated with post-thrombolytic haemorrhagic transformation in an analysis of 70 patients with hyperacute stroke (Kakuda et al., 2005). A further study showed that baseline CMBs were not associated with subsequent haemorrhagic transformation after acute atherothrombotic stroke regardless of thrombolytic therapy (Lee et al., 2008b). These data were pooled in a systematic review and meta-analysis of 790 patients, which revealed a pooled relative risk (RR) of post-thrombolysis ICH associated with CMBs of 1.90 (95% confidence intervals [CI] 0.92-3.93, p=0.082) (Charidimou et al., 2012b). In light of these results, it appears that the presence of CMBs should not be a contra-indication for thrombolysis in patients with acute IS.

3.5 Other clinical manifestations of cerebral microbleeds

CMBs detected on GRE T2*-weighted MRI have generally been considered as not causing overt clinical symptoms; indeed many reports have referred to them as 'silent' or 'asymptomatic' (Kato et al., 2002; Wong et al., 2003). Very few studies have investigated whether they have any independent effect on brain function. This section considers first the potential mechanisms by which CMBs could cause or be associated with neurological dysfunction and then considers CMB in relation to transient neurological symptoms (mainly in the context of CAA), and briefly discusses their association with disability and mortality.

3.5.1 How could cerebral microbleeds cause clinical symptoms?

Histopathological studies show that CMBs are associated with surrounding tissue damage (Fazekas et al., 1999; Schrag et al., 2010; Tanaka et al., 1999; Tatsumi et al., 2008b), so they might be expected to disrupt brain function. However, their absolute size is very small, of the order of less than a millimetre, and at most a few millimetres, in diameter. As discussed in chapter 2, the absolute size of a CMB is magnified by the blooming effect on T2*-weighted or other iron-sensitive images, meaning that the radiological lesions are generally larger than the true extent of the pathology (Schrag et al., 2010). Nevertheless, lacunes are a clearly accepted cause of acute focal deficits (lacunar syndromes) and are generally considered to be small: < 15mm, with the majority being 2-4 mm in diameter (Fisher, 1965). Absolute measurements of CMB size are scarce, but they seem to be rather smaller than lacunes. One study of CAA showed that a cut-off of 5.7 mm best separated CMBs from 'macrobleeds' (Greenberg et al., 2009a), while another on CAA associated with AD showed that CMBs were mostly around 1 mm in diameter (Schrag et al., 2010). Therefore, it might be expected that the development of a single CMB would be less likely than the development of a lacune to produce clinically evident symptoms. However, it is not unreasonable that CMBs could do so if they form rapidly (as has been shown in a recent study in acute stroke, Jeon et al., 2009), and in a functionally strategic location, for example a small eloquent deep nucleus or white matter tract. The very limited evidence that this can happen is discussed below. Another possibility is that rather than disrupting function by direct destruction of tissue, microbleeding could disrupt the activity of surrounding neurons, thus affecting local brain function or connectivity. This idea is supported by recent experimental studies showing that small cortical CMBs can indeed adversely affect the function of nearby neurons, as shown by a reduced or absent neural response adjacent to a

small experimental haemorrhage (50-200 μm) (Cianchetti et al., 2009). Moreover, most CMBs contain haemosiderin, a compound that may affect the electrical activity of the cortex (Baumann et al., 2006). A further possibility is that CMBs are a marker of small vessels with impaired vasoreactivity, which could impair brain function locally or even cause small areas of ischaemia.

More likely perhaps than such local effects, is that the accumulation of multiple CMBs over time could have a more insidious effect on the brain functions that depend on the integrity of widespread anatomical networks, for example cognition or gait. This was discussed in section 1.6.4. This hypothesis is analogous to the proposed mechanism for deficits produced by ischaemic WML, including lacunes, of which the majority do not cause acute stroke syndromes but can cumulatively disrupt cognition or gait (Baezner et al., 2008). This idea is considered later in relation to CMBs and cognition.

A fundamental challenge in studying how CMBs could affect brain function is that they are closely linked to many clinical and imaging manifestations of cerebrovascular disease, including all types of IS and ICH, and WML and lacunes on MRI. It is therefore difficult to determine whether CMBs have any independent functional effects, and well-designed large studies addressing this remain extremely scarce.

3.5.2 Cerebral microbleeds and transient neurological symptoms

To date, only a limited number of case reports or case series addressing a possible link between CMBs and transient neurological symptoms have been published. In these cases, a causal link between the CMB and a transient event was usually suspected because of the strategic location of the CMB in an anatomical area compatible with the focal symptoms. Most of the cases described have been in patients with clinical features of CAA. As discussed above, there are several ways in which CMBs could theoretically be associated with or cause focal symptoms. First, they could form suddenly and by a direct tissue-destructive effect cause neurological symptoms, analogous to the acute focal symptoms caused by lacunar infarction. We recently published 2 cases of patients who developed acute neurological symptoms presumably related to the formation of new CMBs (Teo et al., 2011). In both patients, no acute ischaemic changes were visible on MRI and the new CMB was anatomically correlated with the symptoms (Figure 42). In another report suggesting this as a mechanism, Watanabe and Kobashi described a 72-year-old patient who presented with a lateral gaze disturbance (Watanabe and Kobashi, 2005). A CMB was found

in the contra-lateral pontine medial lemniscus when MRI was undertaken 6 months after the onset of symptoms, with no other lesion seen to account for the clinical presentation (Watanabe and Kobashi, 2005). An MRI 6 months prior to the symptoms had shown ischaemic small vessel changes (multiple lacunar infarcts and leukoaraiosis), but no CMBs. Although the development of the CMB after the onset of symptoms cannot be ruled out, the anatomical correlation between the CMB and the symptoms suggests that a single CMB lesion can cause focal neurological symptoms, but this is probably rare in comparison with symptomatic lacunar infarcts.

A second way in which CMBs might be associated with focal symptoms is by their association with small areas of ischaemia. This possibility has been considered mainly in relation to CAA. Patients with CAA have a propensity to develop ICH, either macroscopic in the form of lobar haemorrhages or microscopic (CMBs). However, recurrent transient neurological symptoms without lobar haemorrhage have also been consistently reported. These symptoms were attributed to recurrent TIAs, a label implying ischaemia as a mechanism in various case reports (Chamouard et al., 1988; Okazaki et al., 1979; Smith et al., 1985; Yong et al., 1992). In support of this possibility, there is histopathological evidence of areas of infarction in severe CAA (Cadavid et al., 2000; Okazaki et al., 1979; Olichney et al., 1995; Wattendorff et al., 1995). Increasing neuroimaging evidence with advanced MRI techniques also suggests that small areas of acute infarction occur in a substantial proportion of patients. Kimberly et al. recently described subclinical ischaemia on diffusion-weighted MRI in 12 out of 78 patients with CAA (Kimberly et al., 2009), and a case report has also described the dynamic evolution of ischaemic areas in CAA (Menon and Kidwell, 2009). Ischaemic lesions were also noted in approximately 20% of patients with CAA in a study that will be described in chapter 5. In the published reports, these lesions seem mostly to be asymptomatic, so may not be a likely cause of acute focal symptoms. The clinical significance of small areas of ischaemia in CAA clearly requires further study.

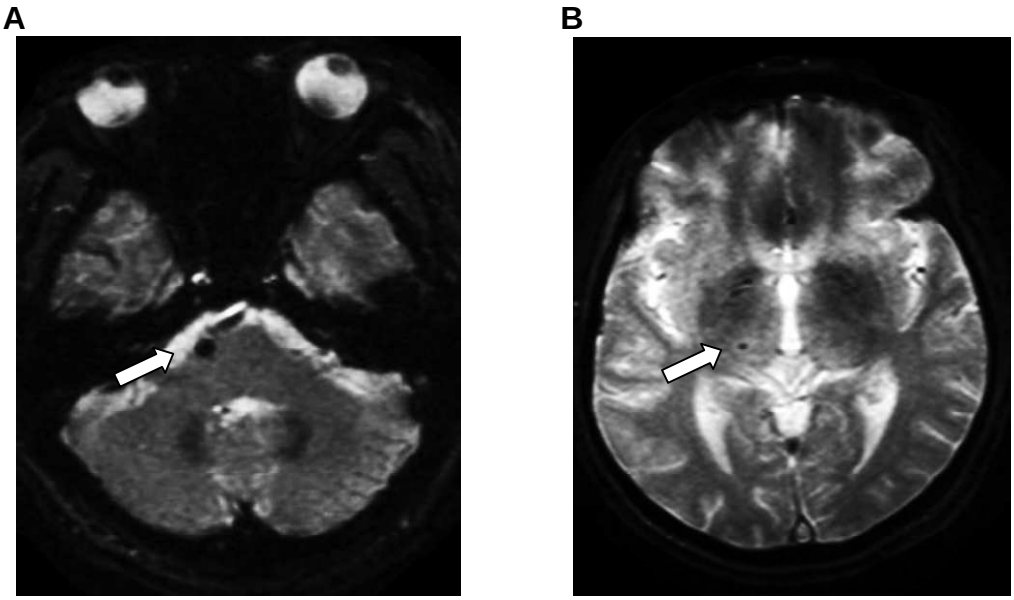


Figure 42. Axial GRE T2*-weighted MR images of 2 patients with acute stroke syndromes presumably caused by CMBs.

A. The image shows a round 6-mm well-circumscribed hypointense lesion in the right pons (white arrow) in a 69 year old hypertensive patient who presented with sudden onset right UMN facial weakness, ataxia and gaze-evoked nystagmus on right gaze. The MRI was performed on the day of presentation. There was no area of restricted diffusion to suggest acute ischaemia. **B**. The sequence shows a 2-mm solitary brain CMB in the ventral posterior nucleus of the right thalamus (white arrow). The 78 year old patient was hypertensive and diabetic, and presented with sudden onset paraesthesia over the left upper limb and cheek. There were no other lesions visible on MRI. From Teo et al., 2011

A third, and perhaps the most likely possibility, is that CMBs may be associated with 'electrical' disturbances of nearby tissue; that is, they may cause cortical spreading depression. Cortical spreading depression is more likely than seizures because seizures are briefer. This possibility is supported by the clinical nature of the transient attacks described in CAA. In a series reported by Greenberg et al. (1993), four patients subsequently diagnosed with CAA presented initially with transient neurological symptoms. These episodes were multiple, and mostly stereotyped, with focal weakness, paresthesias or numbness and a spreading onset, with one patient reporting visual misperceptions. Although in some respects these events resemble TIAs, the gradual onset and positive neurological phenomena are atypical. Interestingly, three of these patients developed subsequent large ICHs in the cerebral territory corresponding to the location of the preceding transient deficits. The similar location involved in the original spells and in the subsequent macrohaemorrhages suggests that in some cases a small lesion (e.g. a CMB) may be a marker for an area of fragile abnormal vessels (perhaps with focal amyloid deposition or microaneurysms) that heralds a larger lobar ICH. Although this hypothesis

could not be tested at that time because CMBs as defined currently were not detectable on the imaging available, T2-weighted and GRE imaging did reveal multiple cortical and subcortical foci of signal loss that correlated anatomically with the symptoms described. More recently, Roch et al. (2005) described six patients with cognitive complaints and recurrent stereotyped episodes of transient motor or sensory symptoms. Imaging with GRE T2*-weighted sequences showed multiple CMBs in three of these patients (of which two were subsequently proven to have CAA on histopathological analysis), while the other patients had a reduced signal in the cortical sulci compatible with haemosiderin deposition and one patient had a thalamic infarct. Four of these patients responded to anticonvulsant drugs, and two improved with the cessation of antiplatelet therapy. The hypothesis that CMBs could cause seizures or seizure-like attacks is also compatible with the observations that haemosiderin, a key component of CMBs on histopathological studies, can be irritant and epileptogenic when close to the cortex (Baumann et al., 2006), and that experimental cortical microhaemorrhages cause functional disturbance of adjacent neurons (Cianchetti et al., 2009). The response of the transient neurological attacks in some patients to anticonvulsant medication is further evidence of a seizure-like mechanism (Roch et al., 2005). It should also be mentioned here that focal convexity atraumatic subarachnoid bleeding is also increasingly recognised as a cause of recurrent stereotyped neurological attacks, described in some cases as rather like migraine auras (Izenberg et al., 2009; Kumar et al., 2010). This pattern of subarachnoid bleeding has also been recognised as a feature of CAA in association with CMBs (Kumar et al., 2010).

In summary, in patients with transient neurological symptoms related to CAA, a seizure-like mechanism may be likely when the symptoms are stereotypical, spread to contiguous cortical regions or resolve with anticonvulsant drugs (Roch et al., 2005). By contrast, stroke-like events with more typically ‘vascular’ symptoms (i.e. ‘negative’ phenomena including weakness or sensory loss) and longer-lasting deficits may be more likely attributable to acute ‘microinfarcts’ or the direct effects of new symptomatic CMBs. These presentations may lead to considerable therapeutic dilemmas, because if microbleeding is causing the problems, then antithrombotic treatment should presumably be avoided, whereas if ischaemia is the dominant mechanism, then antithrombotic drugs may be warranted, and if the attacks are caused by seizure activity then anticonvulsants are the most logical treatment. We present in figure 43 the case of a patient posing such a diagnostic challenge (Figure 43). However, it must be emphasized that these potential mechanisms suggested for symptomatic CMBs are highly speculative: first, due to the nature and characteristics of

CMBs, it has not been demonstrated that CMBs cause acute symptoms; second, the ischaemic nature of the lesions causing acute symptoms cannot be entirely excluded; finally, the acute symptoms observed could be due to other manifestations of small vessel disease such as WML or CAA. It has to be acknowledged here that many of the manifestations described above are similar to manifestations caused by WML or small cortical infarcts. Spreading depolarisations are possible, as they can be detected using EcoG or CASL, but are usually associated with major brain injury (e.g. trauma, malignant infarct or large SAH). Imaging that precedes symptoms onset is lacking in many of the reports, and even with pre-symptoms imaging, the nature of the lesion described is controversial. With the use of modern MRI techniques, including GRE T2*-weighted, susceptibility-weighted and diffusion-weighted sequences, it should be possible to differentiate symptomatic ischaemic lesions from CMBs with more accuracy, which should increase understanding of transient focal attacks in CAA and allow the most rational and safe management.

It is important to keep in mind that the limited evidence available suggests that new CMBs do not usually seem to cause acute symptoms. This has been shown in a few longitudinal MRI-correlated observational studies in different patient cohorts. New foci of haemosiderin deposition were detected in 38% of patients with lobar haemorrhage (presumed from CAA) followed up over 1.5 years, but all appeared to be clinically silent (Greenberg et al., 1999). In the study reported in chapter 6 of this thesis a cohort of patients were followed up after stroke and TIA at a mean interval of 5.5 years, and found that 50% of those with baseline CMBs had developed new CMBs, compared with only 8% of the CMB-free matched controls, despite most surviving patients remaining clinically stable (Gregoire et al., 2010a). In a recent prospective observational study of 19 patients who underwent cardiac valve surgery, 12 developed new lesions, seen by GRE T2*-weighted sequences, that corresponded to CMBs (Jeon et al., 2010), and only two of these developed transient neurological deficits. In one patient with generalised seizure, facial weakness, irritability and confusion, diffusion-weighted image lesions were found and the relevance of the new microbleeding was unclear. In two other longitudinal studies, no follow-up clinical information about neurological events was reported; one of these studies was in a patient cohort with consecutive IS (Jeon et al., 2009), the other in a longitudinal study of patients in a memory clinic (Goos et al., 2010).

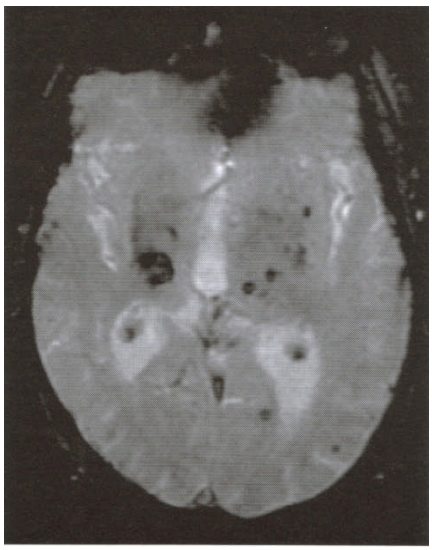


Figure 43. Axial GRE T2*-weighted MR image from a patient who presented with recurrent TIA-like events despite increasingly aggressive antithrombotic treatment.

When antithrombotic therapy was reduced in intensity, the frequency of events was also reduced, suggesting that the antithrombotic treatment may have exacerbated symptoms related to microbleeding. There are multiple lobar and deep CMBs.
Courtesy of Dr Rolf Jäger, National Hospital for Neurology and Neurosurgery, London, UK.

3.5.3 Cerebral microbleeds, disability and death

The accumulation of CMBs (as well as other cerebrovascular disease markers, including leukoaraiosis or lacunes) might reflect progressive small vessel pathology and so could result in the development of progressive disability (including cognitive dysfunction or reduced mobility) or even be associated with an increased risk of death. Data on this are limited, but a prospective study of 94 patients with spontaneous lobar ICHs showed that the higher the number of baseline CMBs, the higher the 3-year cumulative risk of disability or death (Greenberg et al., 2004b). In individuals with more than six CMBs (detected by GRE T2*-weighted imaging), the cumulative risk of the combined endpoint of cognitive impairment, functional dependence or death was 52% (Greenberg et al., 2004b). The occurrence of CMBs has also been associated with clinical disability in the hereditary small vessel disease CADASIL (Lesnik Oberstein et al., 2001; Viswanathan and Chabriat, 2006). The OR for functional dependence (defined as a modified Rankin score of ≥3) per additional CMB was 1.16 (95% confidence internal, 1.01-1.34, p = 0.034) after adjustment for confounding variables (Viswanathan and Chabriat, 2006). In a memory clinic population, CMBs had a strong positive predictive value with respect to mortality in adjusted analyses, particularly when numerous CMBs were present (Henneman et al., 2009).

3.6 Conclusions

To summarise, although there are plausible mechanisms by which CMBs cause clinical symptoms, the evidence that CMBs independently affect brain function remains limited. Case reports suggest that CMBs may be a cause of transient neurological attacks, particularly in patients with CAA. These can mimic TIAs or ISs, but often seem to be atypical, with gradual onset similar to that seen in partial seizures; they may, therefore, result from 'electrical' activity related to CMBs. The evidence that CMBs directly cause focal neurological symptoms through associated tissue damage is even scarcer, but the increasing ability to detect CMBs, together with other advanced neuroimaging methods, should help to determine how important this is as a cause of cerebrovascular events. Some prospective data indicates that new CMBs do not cause obvious symptoms, yet CMBs have been shown to have an independent prognostic significance for disability and mortality in different cohorts of patients, including CADASIL, spontaneous lobar haemorrhages and memory clinic populations. The independent contribution of CMBs to disability and death in other groups, including stroke patients, requires further investigation.

3.7 Thesis aims

Despite the high prevalence of CMBs and the increasing number of publications on the subject, there remains significant uncertainty about the question of whether CMBs have an effect on the human brain and if so, about the mechanisms of this effect. Their discovery in clinical practice causes clinicians anxiety about a number of important questions, for example, what is (1) the reliability of one's ratings of CMB presence and number, and the relative power of the method used to detect them, (2) the clinical significance of their number and distribution in regard to the underlying vasculopathy, (3) the risk of CMB accumulation over time and (4) the risks of subsequent stroke or cognitive impairment in relation to other risk factors of cerebrovascular disease. The answers to these questions will help in increasing our understanding of CMBs, and beyond this, help to disentangle small vessel disease mechanisms and clinical implications. Moreover, it is possible that CMBs as a biomarker of the presence of small vessel disease, may contribute to monitoring the severity of small vessel diseases in the brain, and the impact of preventive measures on small vessel disease progression.

The main objective of this thesis is to provide new insights into the detection of CMBs and their clinical impact in patients with stroke and TIA using neuroimaging studies in a hospital-based setting. This is accomplished by

(1) developing and validating a standardised method of rating CMBs on clinical MR images; this method will be compared with existing method of rating CMBs. Rating CMBs in the brain may be difficult because of the many other lesions seen on MRI with similar morphological or signal characteristics (CMB 'mimics'). The use of a standardised rating method and an appreciation of the distinctive radiological characteristics of CMBs should make this process more reliable. In **Chapter 4**, we developed a new visual rating scale – the Microbleed Anatomical Rating Scale (MARS) – to address some of the challenges of defining and mapping CMBs in the brain.

(2) investigating the important question of the risk of ICH associated with the presence of CMBs. In **Chapter 5,** we explore whether the burden or distribution of CMBs are associated with antiplatelet-related ICH in a case-control and case-case comparison study. This risk could overweight the benefits of antiplatelet therapy in certain categories of patients, especially those taking antiplatelet agents in primary prevention, and those with numerous and lobar CMBs.

(3) examining the dynamic evolution of CMBs in a stroke clinic population and assess risk factors for development of new CMBS. Among these factors, hypertension may play a crucial role and this would suggest a possible role of CMBs as a biomarker of hypertensive injury to the brain and a way to monitor new treatment strategies. In **Chapter 6**, we report the findings of a serial GRE T2* MRI study conducted in a cohort of IS and TIA patients over more than 5 years of follow-up.

(4) investigating whether and how CMBs relate to cognition in a hospital ischaemic stroke and TIA cohort. At the time of writing, the role of CMBs in ischaemic cerebrovascular disease remained largely unexplored. The findings of a large, hospital-based cross-sectional study of the association between CMBs and cognitive impairment are presented in **Chapter 7.1**.

(5) assessing whether CMBs are associated with future cognitive impairment in a cohort of patients with IS and TIA. In **Chapter 7.2**, we formulated the hypothesis that CMBs, as a marker of small vessel pathology, are associated with executive impairment in a stroke clinic cohort and compare the result of their detail cognitive assessment at baseline and 5.7 years follow-up.

(6) exploring how CMBs relate to 'silent' ischaemic lesions in ICH. Emerging data suggest that CAA, a common cause of lobar haemorrhage, is associated with small microinfarctions. CMBs and other markers of ischaemic small vessel diseases might predict silent ischaemia after ICH, especially after lobar ICH secondary to CAA, with implications for the understanding of the mechanisms of small vessel disease. There is increasing evidence that ischaemia and haemorrhage both occur in small vessel disease in a dynamic interplay (**Chapter 8**).

PART 2 – CEREBRAL MICROBLEEDS: NEW INSIGHTS FROM NEUROIMAGING AND CLINICAL STUDIES

Chapter 4 - Exploring Ways of Improving Microbleed Identification, Detection and Reporting

4.1 Cerebral microbleed quantification and mapping

CMBs on gradient-recalled echo (GRE) T2*-weighted MRI may be a useful biomarker for bleeding-prone small vessel diseases, with potential relevance for diagnosis, prognosis (especially for antithrombotic-related bleeding risk), and understanding mechanisms of symptoms, including cognitive impairment. To address these questions, it is necessary to reliably measure their presence and distribution in the brain. At the time of writing, some previous studies had reported information on the reliability of their CMB ratings, but only one had provided the characteristics of the instrument used in a study that validated the first published visual CMB rating scale (Brain Observer Micro Bleed Scale [BOMBS], Cordonnier et al., 2009). We designed and systematically validated our own scale, the MARS. We measured intra-rater and inter-rater agreement for presence, number and anatomic distribution of CMBs using MARS across 2 different MRI sequences (TE = 40 ms and TE = 26 ms) in a representative stroke population. We compared the characteristics of MARS and BOMBS, and tested their inter-rater agreement on the same population sample to compare their reliabilities. We finally made recommendations for future users of visual CMB rating scales, to allow the combination of reliable data across different centres, and develop clear imaging standards for CMB rating.

4.2 Rationale for the use of standardised visual rating scales for cerebral microbleeds

Like WML (leukoaraiosis) (Hachinski et al., 1987), CMBs are recognised as an MRI correlate of small vessel pathology. Over many years, WML on MRI have been extensively investigated, and a number of rating scales have been developed to assess their severity and location (Scheltens et al., 1998). These have been shown to have good inter-rater reliability and continue to be widely used. While volumetric quantification of WML is considered superior to rating scales in assessing the burden of disease, rating instruments continue to be used because of their practicality and applicability to standard clinical datasets. Since awareness of CMBs is much more recent, few standardised instruments have yet been developed, and there has been great inconsistency in the methods and reporting of reliability in CMB studies to date. Investigators have used a wide variety of MRI sequences and rating methods for CMBs, the characteristics of which varied greatly or were inconsistently reported. The level of observer agreement varies considerably across recent

studies. A summary of the differences in MRI specifications, brain CMB size definitions and reports of inter- and intra-observer reliability in recent cohort studies (published in 2009) is shown in Table 10. In 13 out of 21 studies (62%), there was no report of the reliability of their CMB measures. Variations in methods included differences in the properties of the rating scales, in MRI sequences characteristics, in CMB defining criteria and in observers' training background (e.g. neurology or radiology) or MRI rating experience (Cordonnier et al., 2007; Greenberg et al., 2009b). MRI sequence characteristics, especially the TE, can affect the number of lesions classified as CMBs (Gregoire et al., 2010b). The inter-observer agreement varied from a kappa factor of 0.68 to one of 0.97 in these studies. In general, agreement about the number of CMBs is higher than that for presence of CMBs (data not shown). Because of the heterogeneity in these studies, the data cannot be easily compared and interpreted (Gregoire et al., 2009). A CMB rating scale would be a useful tool to overcome these issues; it would provide a uniform rating methodology (including clear definitions of CMB criteria and anatomical regions) and enable reliable (reproducible) data collection to allow more informative cross-study comparisons.

4.3 Presentation of the Microbleed Anatomical Rating Scale

4.3.1 Study design and methods

We developed MARS, an anatomically detailed scale designed to be reliable, easy to use and generalisable across MRI sequences and observers with various imaging experience (Figure 44). We specifically included a categorisation of CMBs into individual brain lobes, since this anatomical information may be important to assess their contribution to VCI or diagnosing CAA (Cordonnier et al., 2006; Hachinski et al., 2006; Werring et al., 2004; Seo et al., 2007). Indeed, the MARS was developed from earlier basic rating schemes originally employed in studies of cognition (Werring et al., 2004). We systematically measured the intra- and inter-rater reliabilities of the scale for CMB presence and number in lobar, deep and infratentorial regions, in a representative stroke population.

Population

We considered unselected, consecutive patients (N=426) admitted to the Stroke Service at the National Hospital for Neurology and Neurosurgery (NHNN) from July 2004 to October 2007. The Stroke Service takes all suspected stroke patients admitted from the surrounding district and has a policy of performing MRI with GRE T2* sequence in all of them unless there is a contra-indication (e.g. too medically unstable, severe claustrophobia, metallic implants). Patients who did not have an MRI were excluded. We excluded patients without GRE T2*-weighted MRI of sufficient quality for analysis (e.g. due to motion artefact).

Standard protocol approvals

This study on human subjects received approval from the NHNN and Institute of Neurology Joint Research Ethics Committee (approval number 07/Q0512/39; date of approval 22 October 2007).

Imaging protocols

All MRIs were carried out at 1.5 Tesla field strength using two MRI systems. The majority of patients (N=271) was imaged on GE Medical Genesis Signa system using a TE of 40ms for the T2*-weighted sequence. The parameters of the sequences were as follows: axial T2-weighted FSE (TR 6000, TE 105, FA 90, matrix 256x224, FoV 24x18, slice thickness 5 mm, slice gap 1.5mm, NEX 2); axial GRE T2* (TR 300, TE 40, FA 20, FoV 24x18, matrix 256x160, slice thickness 5mm, slice gap 1.5mm, NEX 1). A smaller number of patients (N=30) were

imaged on a Siemens Avanto system using a TE=26ms for the T2*-weighted sequence. The parameters of the sequences were as follows: axial T2-weighted FSE (TR 4320, TE 106, FA 150, matrix 448x392, FoV 24x18, slice thickness 5mm, slice gap 1.5mm, NEX 2); axial GRE T2* (TR 800, TE 26, FA 20, FoV 24x18, matrix 512x448, slice thickness 5mm, slice gap 1.5mm, NEX 1).

Table 11. Variations in MRI specifications, brain CMB size and results of inter- and intra-rater reliabilities in cohort studies published in 2009

Study	Sample size	Demographics	MRI Parameters					CMBs		Agreement		
			TE (msec)	TR (msec)	Magnet Strength (Tesla)	Slice thickness (mm)	Slice gap (mm)	MB size (mm)	Raters	IE	IA	Characteristic
Lee et al., 2009	24	Warfarin users	15	500	1.5	6	2	<5	2 Neu	0.88	-	Presence
Henneman et al., 2009	1138	Memory clinic	15-22	600-800	1.0/1.5	5	1	<10	3, trained	>0.90	>0.90	Number
Nandigam et al., 2009	20	CAA	24-25	750-763	1.5-3.0	1.5-5	0-1	NR	2 raters	0.97	0.8-0.9**	Number
Igase et al., 2009	377	Healthy	NR	NR	3.0	NR	NR	<5	2 Neu	'very good'	-	-
Staekenborg et al., 2009	152	MCI	22	800	1.0	5	1.5	2-10	NRa	-	-	-
Henskens et al., 2009	192	Hypertensive	23	736	1.5	5	0.5	<5	2 Neu	0.68	-	Presence
Kirsch et al., 2009	73+33	MCI+healthy	18	500	1.5	4	NR	<=10	4 'readers'	-	-	-
Tang et al., 2009	519	Acute IS	30	350	1.5	5	0.5	2-10	1 Neu	0.78	0.85	Presence + Number
van Rooden et al., 2009	27	ICH with HCHWA-D	45-48	2593-3070	1.5	6	0.6	<5-10	1 NRa	-	-	-
Nishikawa et al., 2009	698	No previous clinical event	23	889	1.5	6	1	<10	1 NRa, 1 NSu	-	-	-
Cho et al., 2009a	152	Acute IS	20	700	1.5	5	2	<10	2 Neu	0.881	0.881	-
Staals et al., 2009	123	First-ever lacunar stroke	23	shortest	1.5	5	0.5	<10	2 Neu	0.68	-	Presence
Ishikawa et al., 2005	106	ICH	23	889	1.5	6	1	<10	1 Ra, 1 NSu	-	-	-
Lim and Kim, 2009	234	Primary ICH	20	425	3.0	5	2	<5	1 NRa	-	-	-
Sun et al., 2009	998	Acute IS	30	300	1.5	5	0.5	2-10	NRa	-	-	-
Jeon et al., 2009	237	Acute IS	30	400	1.5	5	2	≤5	2 raters	-	-	-
Klein et al., 2009	60	Infective endocarditis	17.3	750	1.5	5	0.5	≥5, >5 - ≤10	2 NRa	-	-	-
Orken et al., 2009	141	IS on Warfarin	15	640	1.5	NR	NR	<5	2 raters	-	-	-
Goos et al., 2009	63	Alzheimer disease	22-25	415-800	1 + 1.5	5	1-1.5	≤10	NRa	-	-	-
Park et al., 2009	21	Mild TBI without ICH	26	800	1.5	5	2	<5	NRa	-	-	-

IE = Inter-rater; IA = intra-rater; MCI = mild cognitive impairment; CAA = cerebral amyloid angiopathy; IS = Ischaemic stroke; HCHWA-D = hereditary cerebral haemorrhage with amyloidosis-Dutch type; TBI = Traumatic brain injury; Neu = Neurologist; NRa = Neuroradiologist; NSu: Neurosurgeon; Ra: Radiologist; NR = not reported

Brain cerebral microbleed rating

We tested the reliability of the scale for CMBs presence and number in all individual cerebral regions in the patients scanned at TE=40ms. The images of the patients scanned at TE=26ms were studied in a secondary analysis for presence of CMBs only. Images were displayed using the Agfa IMPAX picture archiving and communications system (PACS) (Agfa, Mortsel, Belgium) on 3-megapixel premium diagnostic grayscale displays (Barco [Kortrijk, Belgium] Coronis 3MP MDCG-3120-CB), and assessed by a clinical neurologist (SMG) and trainee neurologist (UJC) in semi-dark conditions. Rater 1 (SMG) had five years' experience in neuroimaging; rater 2 (UJC) had one year's experience. Rater 2 rated the MRIs twice at a 4 week interval, chosen as the minimum time likely to be used in prospective studies. Rater 1 rated the MRIs twice at a 1 year interval on a subsample of the first 100 consecutive patients. Both raters had received training sessions in CMB detection from a senior neuroradiologist (HRJ). Each rater was blinded to clinical data and the other rater's ratings. Cases of disagreement were reviewed by a consultant vascular neurologist (DJW) and consultant neuroradiologist (HRJ), both with extensive experience in CMB rating. Appropriate guidance was incorporated into instructions for future users of the scale.

The microbleed anatomical rating scale

We classified CMBs into 'definite' and 'possible' categories because a previous study suggested that such classification improves reliability (Cordonnier et al., 2009). Definite CMBs were defined as small, rounded or circular, well-defined hypointense lesions within brain parenchyma with clear margins ranging from 2 to 10 mm in size on GRE T2*-weighted images; possible CMBs were less well-defined, less hypointense, or not strictly rounded or circular. The 2-10mm size range includes the highest upper limit defined in previous studies (Table 10) (Cordonnier et al., 2007). We included a lower size limit in accordance with consensus guidelines on standards for neuroimaging in VCI (Hachinski et al., 2006). CMB mimics were carefully excluded using all available imaging. In the basal ganglia we considered strictly unilateral lesions without evidence of corresponding infarction (on T2-weighted and FLAIR images) or calcification (on CT scans) to be definite.

CMBs were classified into deep, lobar and infratentorial categories. Lobar MRI landmarks were defined according to Stark and Bradley (1999), and included cortical and subcortical regions (including subcortical U-fibres). Deep regions included the basal ganglia, thalamus, internal capsule, external capsule, corpus callosum and deep and periventricular white

matter (DPWM); infratentorial regions included the brainstem and cerebellum. All regions were presented for easy reference in an anatomical diagram (drawn by SMG using representative axial MR images) adjacent to the scale. DPWM was defined as white matter adjacent to or within approximately 10 mm of the lateral ventricular margin. Definite and possible CMBs were reported at each location on the MARS rating form (Figure 44). The sum of definite and possible CMBs was recorded as total CMBs.

Statistics

Intra- and inter-rater agreements for the presence or absence of CMBs were calculated using the non-parametric un-weighted κ measure of agreement. κ results were interpreted as poor (0-0.20), fair (0.21-0.40), moderate (0.41-0.60), good (0.61-0.80) or very good (0.81-1) agreement as per Landis and Koch (1977). We used the intraclass correlation coefficient to assess the intra and inter-rater reliabilities for the number of CMBs. The intra-rater reliabilities of each rater were compared. All statistical tests were conducted using the Statistical Package for the Social Sciences (SPSS) for Windows version 16.0 (SPSS Inc., Chicago, IL).

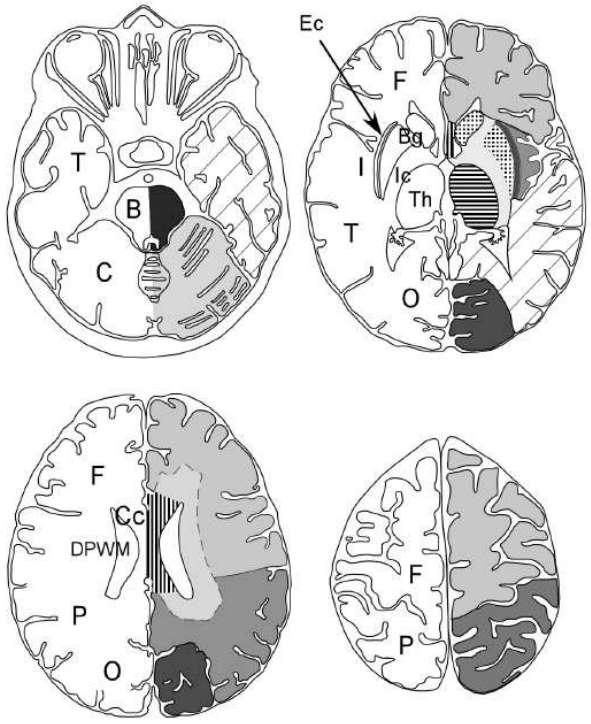
The Microbleed Anatomical Rating Scale

Patient ID: Date of Birth _ _/_ _/_ _ _ _ Date of MRI _ _/_ _/_ _ _ _

DEFINITE MICROBLEEDS: Small, round, well-defined, hypointense on GRE T2*; 2-10 mm; not well seen on T2

MICROBLEED MIMICS
- Vessels: linear / curvilinear lesions in subarachnoid space, usually cortical or juxta-cortical (visible on T2)
- Mineralization in globi pallidi or dentate nuclei: symmetrical hypointensities (may be bright flecks on CT)
- Haemorrhages within area of infarction (look at the T2, FLAIR or DWI sequences to identify infarction)
- Air-bone interfaces: frontal / temporal lobes (check adjacent GRE T2* slices to clarify)
- Partial volume artifact at the edges of the cerebellum (check adjacent GRE T2* to clarify)
- Small haemorrhages close to a large ICH (visible on GRE T2*) or to an infarct (visible on T2, FLAIR or DWI)

		Right	Left	Right	Left
		DEFINITE		POSSIBLE	
		R	L	R	L
Infratentorial TOTAL	Brainstem (B)				
	Cerebellum (C)				
Deep TOTAL	Basal Ganglia (Bg)*				
	Thalamus (Th)				
	Internal Capsule (Ic)				
	External Capsule (Ec)				
	Corpus Callosum (Cc)				
	Deep and periventricular WM (DPWM)				
Lobar** TOTAL	Frontal (F)				
	Parietal (P)				
	Temporal (T)				
	Occipital (O)				
	Insula (I)				
	TOTALS				



* (Caudate, Lentiform), **Lobar regions include cortex and subcortical white matter

Figure 44. Microbleed anatomical rating scale rating form

4.3.2 Results

Demographic findings

MRI was performed in 355 consecutive patients (83%) admitted to the stroke unit. Fifty-four patients were excluded from the final analysis for insufficient quality MRI due to motion artefact (N=43), or absence of GRE T2*-weighted MRI sequence (N=11). The final cohort consisted of 301 patients, including 271 patients scanned at TE=40ms and 30 patients scanned at TE=26ms. This is summarised in the patient flow diagram (Figure 45). The radiological findings of our patients included ischemic stroke (N=109, 36%), small vessel disease with acute infarction (N=106, 35%), small vessel disease without acute infarction (N=28, 10%), ICH (N=15, 5%), ICH with small vessel disease (N=12, 4%) and SAH (N=9, 3%). Thirteen subjects (4%) had a normal intracranial MRI scan; 9 subjects (3%) had another pathology. One hundred seventy-two were male, 129 were female; mean age was 65.2 years (range 18-97). CT was available in the majority of patients (94%, N=283).

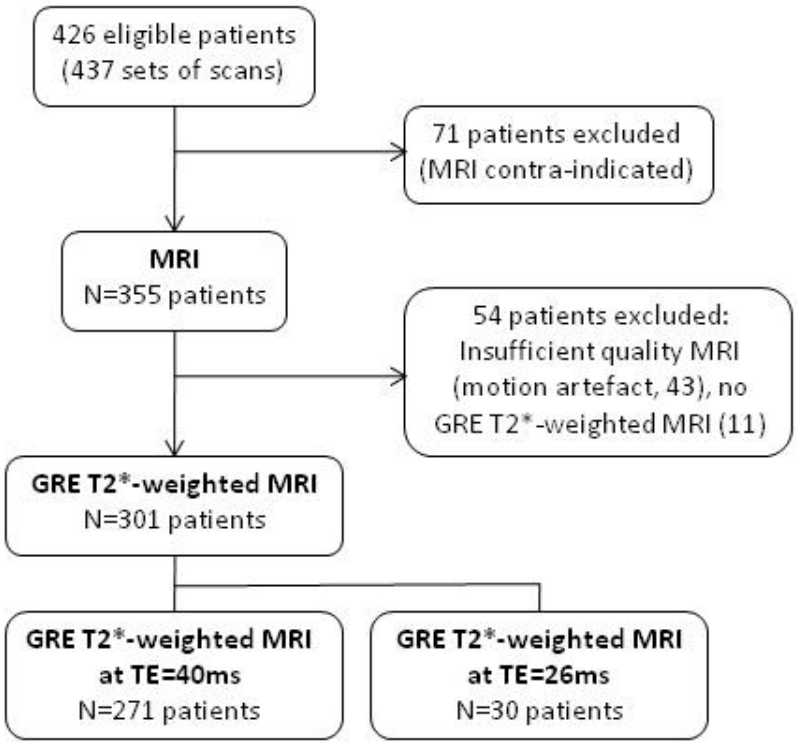


Figure 45. Patient flow diagram of MARS study

Cerebral microbleeds in any location of the brain

CMBs were most prevalent in the lobar region (17-22%) followed by deep (14-15%) and infratentorial regions (6-12%). Results of intra- and inter-rater reliabilities for the presence and number of definite, possible and total CMBs in the brain are shown in Table 11. Raters disagreed on the presence or absence of one or more definite CMBs in 69 cases (25%), of which 28 (41%) were thought to have a single definite CMB by at least one of the raters. Because the clinical and pathophysiological significance of a single CMB is uncertain, we investigated the effect of excluding patients with one definite CMB from the analysis. After excluding these patients, there was a substantial improvement of the inter-rater agreement for the presence of definite CMBs in any location (κ = 0.91 [95% CI 0.85-0.97]). The intra-rater reliability of rater 1 for the presence of CMBs in all locations at a 1-year interval was good (κ = 0.75 [95% CI 0.61-0.89], but lower than that of rater 2 at a 4 week interval (κ = 0.89 [95% CI 0.79-0.99]).

Cerebral microbleeds in lobar, deep and infratentorial regions

CMBs were most prevalent in the lobar region (17-22%) followed by deep (14-15%) and infratentorial regions (6-12%). Intra- and inter-rater reliabilities for the presence and number of definite CMBs in lobar, deep and infratentorial regions were good to very good (Table 11).

Cerebral microbleeds in individual anatomical regions

For the presence of CMBs, intra-rater agreement was good to very good in all individual regions. Inter-rater agreement was good to very good for the presence of CMBs in individual lobes, in the thalamus and brainstem; moderate inter-rater agreement was obtained in the cerebellum and basal ganglia. All inter- and intra-rater reliabilities for CMB presence and number are shown in Table 11. We could not calculate the reliability for CMBs in the insula and in deep locations other than thalamus and basal ganglia because of the small number of patients with CMBs in these regions.

The reliability of the scale for possible and total CMBs was lower than that for definite CMBs in all regions of the brain (Table 11).

Table 12. Reliability of MARS at TE=40ms for presence and number of CMBs (kappa agreements and correlation coefficients)

CMBs	Presence of CMBs*				Number of CMBs**			
	N (%), rater 1	N (%) rater 2	IA (95% CI)	IE (95% CI)	N (range) rater 1	N (range) rater 2	IA (95% CI)	IE (95% CI)
Any location								
Definite	67 (25)	87 (32)	**0.85 †** (0.77-93)	**0.68 †** (0.58-0.78)	266 (0-23)	360 (0-29)	0.98 (0.97-0.99)	0.93 (0.91-0.94)
Possible	52 (19)	63 (23)	**0.72 †** (0.62-0.82)	0.31 § (0.17-0.45)	109 (0-14)	147 (0-22)	0.86 (0.83-0.89)	0.58 (0.49-0.65)
Total	84 (31)	105 (39)	**0.84 †** (0.78-0.90)	**0.61 †** (0.51-0.71)	375 (0-35)	507 (0-49)	0.98 (0.97-0.98)	0.91 (0.89-0.93)
Lobar, deep and IT								
Definite								
Lobar	46 (17)	60 (22)	**0.85 †** (0.77-0.93)	**0.72 †** (0.62-0.82)	156 (0-14)	209 (0-22)	0.98 (0.97-0.98)	0.91 (0.89-0.93)
Deep	37 (14)	40 (15)	**0.94 †** (0.88-1)	**0.71 †** (0.59-0.83)	83 (0-8)	97 (0-9)	0.97 (0.96-0.98)	0.92 (0.90-0.94)
IT	16 (6)	32 (12)	**0.85 †** (0.75-0.95)	**0.64 †** (0.48-0.80)	27 (0-8)	54 (0-6)	0.91 (0.88-0.93)	0.73 (0.67-0.78)
Possible								
Lobar	32 (12)	40 (15)	**0.76 †** (0.64-0.88)	0.39 § (0.23-0.55)	66 (0-14)	95 (0-20)	0.89 (0.86-0.91)	0.72 (0.66-0.78)
Deep	19 (7)	20 (7)	0.56 § (0.36-0.76)	0.20 § (0-0.40)	34 (0-12)	23 (0-2)	0.47 (0.37-0.56)	0.16 (0.05-0.28)
IT	9 (3)	22 (8)	0.54 § (0.34-0.74)	0.22 § (0.02-0.42)	9 (0-1)	29 (0-3)	0.97 (0.97-0.98)	0.145 (0.03-0.26)
Total								
Lobar	60 (22)	74 (27)	**0.84 †** (0.76-0.92)	**0.64 †** (0.54-0.74)	222 (0-28)	304 (0-42)	0.96 (0.94-0.96)	0.91 (0.89-0.93)
Deep	49 (18)	50 (18)	**0.87 †** (0.79-0.95)	**0.67 †** (0.55-0.79)	117 (0-17)	120 (0-10)	0.85 (0.81-0.88)	0.88 (0.85-0.91)
IT	23 (8)	46 (17)	**0.80 †** (0.70-0.90)	0.56 § (0.42-0.70)	36 (0-8)	83 (0-6)	0.85 (0.81-0.88)	0.66 (0.58-0.72)
Individual lobes and regions (definite CMBs)								
Frontal	24 (9)	24 (9)	**0.89 †** (0.79-0.99)	**0.77 †** (0.63-0.91)	41 (0-4)	53 (0-7)	0.95 (0.93-0.96)	0.84 (0.80-0.87)
Temporal	22 (8)	36 (13)	**0.87 †** (0.77-0.97)	**0.73 †** (0.59-0.87)	53 (0-5)	80 (0-9)	0.96 (0.95-0.97)	0.81 (0.77-0.85)
Parietal	21 (8)	26 (10)	**0.98 †** (0.94-1)	**0.74 †** (0.60-0.88)	35 (0-7)	49 (0-5)	0.93 (0.92-0.95)	0.75 (0.69-0.79)
Occipital	13 (5)	15 (6)	**0.70 †** (0.50-0.90)	**0.62 †** (0.40-0.84)	24 (0-4)	19 (0-3)	0.75 (0.69-0.79)	0.65 (0.58-0.72)
Insula	3 (1)	7 (3)	**0.85 †** (0.65-1)	ND	3 (0-1)	8 (0-2)	0.89 (0.87-0.92)	ND
Thalamus	20 (7)	27 (10)	**0.96 †** (0.90-1)	**0.84 †** (0.72-0.96)	44 (0-6)	57 (0-8)	0.95 (0.94-0.96)	0.91 (0.88-0.93)
Basal ganglia	18 (7)	16 (6)	**0.87 †** (0.75-0.99)	0.44 § (0.22-0.66)	23 (0-4)	26 (0-4)	0.88 (0.85-0.91)	0.59 (0.51-0.66)
Other deep locations[‡]	13 (5)	8 (3)	ND	ND	16 (0-2)	14 (0-3)	ND	ND
Brainstem	7 (3)	11 (4)	**0.95 †** (0.85-1)	**0.77 †** (0.55-0.99)	8 (0-2)	15 (0-4)	0.96 (0.95-0.97)	0.76 (0.71-0.81)
Cerebellum	11 (4)	27 (10)	**0.81 †** (0.69-0.93)	0.55 § (0.43-0.67)	19 (0-7)	39 (0-5)	0.86 (0.83-0.89)	0.73 (0.66-0.78)

* un-weighted kappa statistic, ** intraclass correlation coefficient

† = Good/very good agreement §= Moderate agreement ‡ Internal capsule, external capsule, corpus callosum, deep and periventricular white matter

MARS = Microbleed Anatomical Rating Scale; TE = echo time; CI = confidence interval; IA: intra-rater; IE: inter-rater; IT: infra-tentorial; ND: not done

Agreement for cerebral microbleed rating at TE=26 ms

At TE=26ms (N=30), we obtained very good intra- and inter-rater agreements for the presence of definite CMBs (intra-rater κ = 1 and inter-rater κ = 0.87 [95% CI 0.61-1]) in any location of the brain.

4.3.3 Discussion

With the increasing interest in the clinical relevance of CMBs in individuals with cerebrovascular disease or dementia and in normal ageing, there is a need for a reliable instrument to rate their presence, number and distribution throughout the brain. Although some previous studies have reported information on the reliability of CMB rating (Copenhaver et al., 2008; Gorner et al., 2007; Henneman et al., 2009; Henskens et al., 2008; Lee et al., 2008b; Lee et al., 2009; Nandigam et al., 2009; Pettersen et al., 2008; Yakushiji et al., 2008), only one (Cordonnier et al., 2009) clearly described the characteristics of the instrument used, and most did not report the intra- and inter-rater reliabilities for all relevant anatomical regions. Here, we have shown that MARS has good-to-very-good intra- and inter-reliability for the presence and number of definite CMBs in individual cerebral lobes, deep and infratentorial regions. There was only moderate agreement for the presence of possible CMBs.

The particularity of MARS is to assess CMBs in individual cerebral lobes, as well as deep regions. Lobar anatomical information may be important for studies investigating the impact of CMBs on cognitive functions in cerebrovascular and degenerative diseases (Hachinski et al., 2006; Schneider, 2007; Werring et al., 2004; Liem et al., 2009; Seo et al., 2007) or for the diagnosis of CAA, in which a preferential parieto-occipital distribution for CMBs has been reported (Greenberg et al., 1996). Indeed, our classification is based partly on the theoretical potential for CMB distribution to help distinguish CAA from hypertensive small vessel disease (Vernooij et al., 2008b). The deep category includes brain structures potentially affected by hypertensive disease of the small penetrating arteries; lobar regions include the cortico-subcortical regions (cortex and subcortical white matter), more likely to be affected by CAA. The use in MARS of a similar lobar scheme to previously validated age-related white matter change scales (Hachinski et al., 2006; Wahlund et al., 2001) allows the investigation of regional correlations between WML, CMBs and clinical factors. MARS has other features designed to maximise ease of use: the rating form includes a convenient summary of the total CMB counts for the whole brain and each anatomical region, and a

clear guide to anatomical boundaries of the cerebral lobes and regions (Figure 44). MARS does not subclassify CMBs according to their size, which in another recently published scale (BOMBS) (Cordonnier et al., 2009) may unnecessarily complicate the ratings without adding extra useful information. A full comparison of the two scales is available in the next section.

The present study has strengths in comparison to some previous reports on CMB rating. First, we systematically tested MARS in a representative stroke population; many previous studies have included healthy populations (Jeerakathil et al., 2004b; Roob et al., 1999b; Yakushiji et al., 2008) or patients with a single cerebrovascular diagnosis (ICH (Greenberg et al., 2004a; Lee et al., 2005; Roob et al., 2000), CAA (Lee et al., 2005; Nandigam et al., 2009). Second, intra-rater reliability has not been addressed with an adequate interval in most previous studies (Sveinbjornsdottir et al., 2008; Vernooij et al., 2008b) but is critically important for longitudinal cohort studies. MARS has shown high agreement with up to a 1 year intra-rater interval, suggesting that it may be useful for such follow-up studies. The lower intra-rater reliability for rater 1 is probably due to the longer interval between ratings compared with rater 2. Third, our study investigated the reliability of quantifying the number of CMBs, which may be relevant in exploring their relationship with other quantitative imaging or clinical data; most previous studies tested the reliability of rating CMB presence but not number (Jeerakathil et al., 2004b; Lee et al., 2009; Lemmens et al., 2007; Seo et al., 2007). Fourth, our results suggest that MARS may be reliable when applied to a range of MRI sequences, in particular across different TE values. The inter-rater reliability in our cohort studied at TE=26ms was better than at TE=40ms; this may be because some CMBs or CMB mimics are missed at TE=26ms, therefore paradoxically increasing the apparent reliability of the scale despite reduced sensitivity. However, we did not repeat MRI studies with different TE on the same patients to investigate this possibility. Previous studies on CMBs reported TE values from 15 to 50ms (Table 10) (Jeerakathil et al., 2004a; Roob et al., 1999b), which may substantially affect the reported prevalence of CMBs (Greenberg et al., 2009b) Long TEs may increase the number and size of CMBs detected (Tatsumi et al., 2008a) but could also reduce image quality and increase unwanted susceptibility artefacts (Greenberg et al., 2009b). Many other MRI acquisition characteristics influence CMB detection, including magnetic field strength, slice thickness, FA and post-processing techniques (including SWI), but the optimum MRI protocol for detecting CMBs needs further study (Greenberg et al., 2009b; Haacke et al., 2007; Scheid et al., 2007; Thomas et al., 2008; Vernooij et al., 2008a). The use of a validated scale could help to identify the MRI acquisition strategy with highest reliability. Finally, our scale had

good reliability for raters with different levels of experience in neuroimaging. Our less experienced rater documented the highest number of CMBs, suggesting that stringency in rating might increase with experience, even when both observers undergo similar training (as in our study). This suggests that to maximise the reliability of CMB rating the same observer should be used for all ratings if possible, even where training is standardised; for multicentre studies, a single rating centre for analysis may also improve reliability. In future studies, automated methods of CMB detection may help to further improve agreement for CMB number.

Regions of the brain that caused discrepancies among our raters and with reduced reliability included the basal ganglia, cerebellum and occipital lobes. In the basal ganglia, susceptibility effects from calcification or iron deposits can mimic CMBs; CT may be helpful to distinguish calcification. In the posterior fossa, causes of disagreement include physiological iron deposits in the dentate nuclei and partial volume artefact from adjacent bony structures. Air-bone interfaces can cause susceptibility artefacts elsewhere, e.g., in the inferior frontal or temporal lobes. Problems from misclassifying CMB mimics can be minimised by careful inspection of adjacent slices and reference to T2, FLAIR and diffusion-weighted images. Because of the lower agreement for possible CMBs, including only definite CMBs may improve the reliability of CMB rating for research studies. Patients with only 1 potential CMB accounted for most cases of disagreement, and inter-rater reliability substantially improved when these patients were excluded. We therefore suggest caution in rating a single CMB as a definite lesion. More work is required to establish whether only patients with multiple CMBs should be included in research studies, and how this affects CMB prevalence in different diseases. This issue is further complicated because the classification of patients into those having single versus multiple CMBs may vary with the MRI technique used: e.g. higher field strengths, thinner slices or SWI seem to increase the conspicuity and number of CMBs detected (Greenberg et al., 2009b).

Our study has some limitations. First, the number of scans at TE=26ms was small (N=30), which may increase the CI for our reliability measures. Second, we chose to investigate the effect of TE on reliability (because TE is likely to have a marked effect on CMB detection), but did not investigate the effect of changing other relevant MRI sequence characteristics (Greenberg et al., 2009b). Both of our observers were neurologists, and it would be of interest to test the reliability of the scale in other types of rater (e.g. radiologists). Finally, there were limitations related to our assessment of reliability. We conducted a limited

assessment of reliability between two raters, and only assessed the reliability of the scale. Other domains of validity need to be established.

4.4 Other standardised cerebral microbleed visual rating scale

4.4.1 BOMBS

At the time of publication of MARS, another visual rating scale had just been published, BOMBS (Cordonnier et al., 2009). In the BOMBS study, pilot evaluations (not using a standardised scale) showed inter-rater agreement for 'certain' CMBs with a kappa value of 0.44, which improved with modifications and standardisation as the BOMBS scale to one of 0.68, tested in a different population to the pilot study. The BOMBS approach did not improve agreement about total CMB counts (certain and uncertain). The BOMBS includes a sub-classification of CMB size as >5 mm or 5-10 mm, although the value of this information in distinguishing CMBs remains unclear (Greenberg et al., 2009a). The BOMBS rating form is presented in Figure 46.

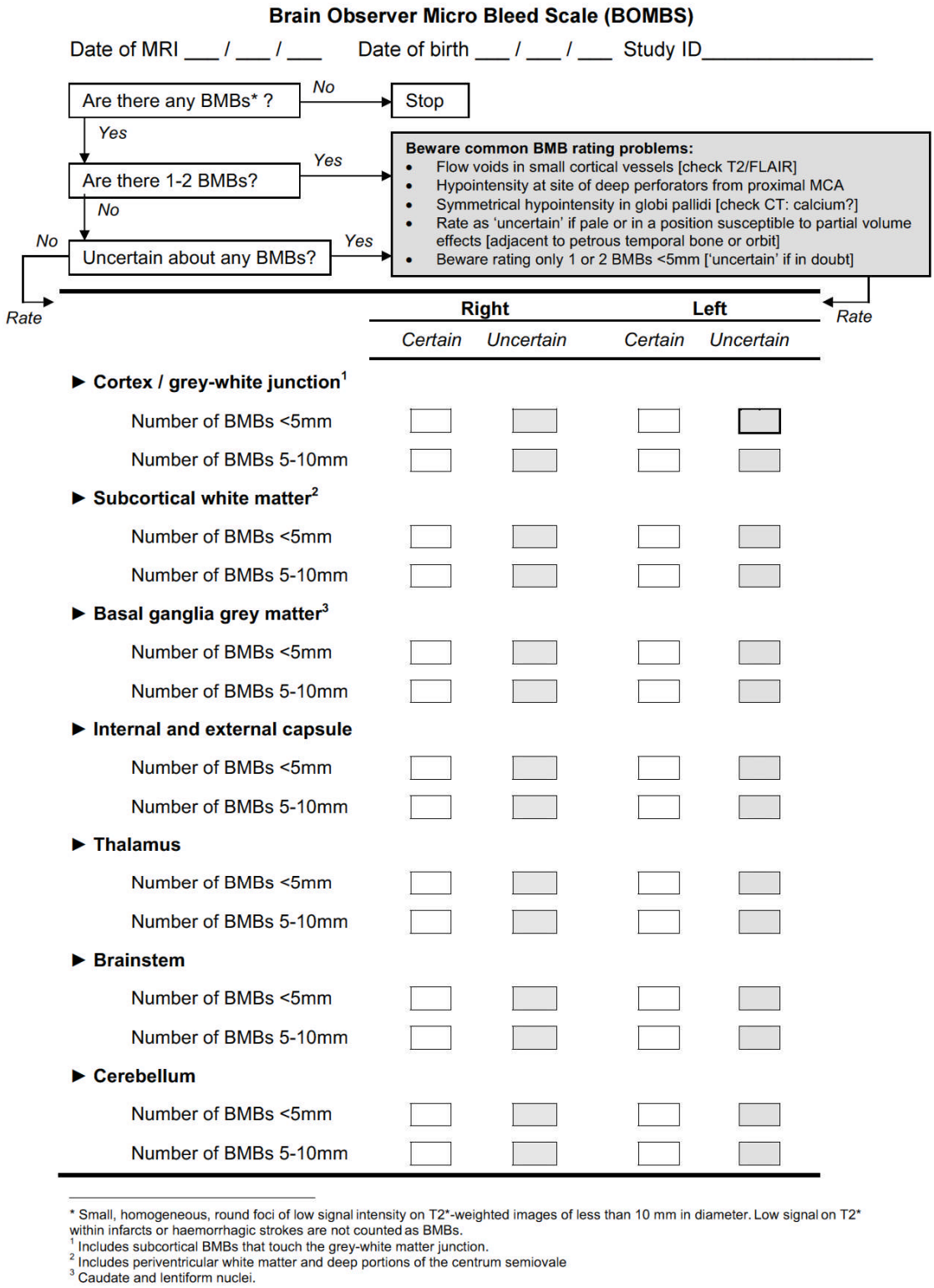


Figure 46. Brain observer microbleed scale (BOMBS)

4.4.2 Comparison between MARS and BOMBS

We performed a limited comparison between BOMBS and MARS by using BOMBS to rate MRI scans from our population to provide a comparison of reliabilities. Both our raters evaluated the first consecutive 100 patients using BOMBS to calculate the inter-rater reliability. The second MARS ratings of both raters were then used to re-calculate its inter-rater reliability for the same sample.

On the sample of the first consecutive 100 patients, the inter-rater reliability of BOMBS for the presence of definite CMBs in all locations was κ = 0.64 [95% CI 0.48-0.80]; the inter-rater reliability of MARS was κ = 0.75 [95% CI 0.61-0.89]. Both MARS and BOMBs have good-to-very-good intra- and inter-rater reliability for CMB presence and number in the brain. Although the 95% CI overlapped, our raters had a higher inter-rater reliability in favour of MARS compared to BOMBS when tested in the same population. The inter-rater reliability for the presence of ≥1 CMB for BOMBS and MARS was generally very similar, though MARS had higher reliability for rating deep CMBs (κ = 0.71; 96% confidence interval [CI] 0.59-0.83 for MARS versus κ = 0.54; 95% CI, 0.25-0.83 for BOMBS), albeit with overlapping CIs. The MARS system was also shown to have very good intra-rater reliability over a 1-year interval (κ = 0.85 for presence of at least one CMB), and high reliability was found using two MRI sequences with different TE values. The testing of rating scales on images collected using different MRI sequences is important because these affect the conspicuity and size of CMBs, which have been shown to influence CMB identification (Nandigam et al., 2009). We acknowledge that the comparison of reliabilities between the two scale was not a validation measure of MARS and was open to inadvertent bias because the same raters undertook the comparisons.

The main difference between BOMBS and MARS is that the latter classifies CMBs into individual lobar anatomical regions, as well as deep structures, with the hypothesis that the lobar location of CMBs is likely to be an important factor in how they might affect brain function (particularly cognition). Both scales provide guidance for use, definition criteria for CMBs and CMB mimics and a table for anatomical categorisation of the CMBs. In each of these scales, there are two steps: first to identify whether a given lesion is likely to be a CMB or not and, second, to record the distribution of CMBs in the brain according to an anatomical scheme. These scales give the option of including ‘definite’ or ‘certain’, as well as ‘possible’ or ‘uncertain’ CMBs. The inclusion of less certain lesions has been shown in both studies to lower the agreement between raters, so it is recommended that, at least

for research studies, only 'definite' CMBs are reported. Furthermore, the clinical relevance of having a single CMB compared with multiple CMBs is not yet determined, though it seems likely that a single CMB has less relevance for clinical impact on brain function or prognosis (Goos et al., 2009). Consequently, in research studies, it is recommended that a distinction is made between patients with just one CMB and those with multiple (>1) CMBs. Also the classification of patients into those having single versus multiple CMBs may vary with the MRI technique used , since MRI acquisition characteristics (including field strength, slice thickness or SWI) have a great influence on CMB detection and identification (Greenberg et al., 2009b).

4.5 Conclusions

There is increasing interest in the use of biomarkers to detect and measure the risk of developing diseases, and to measure disease progression and responses to treatment. Since CMBs can be readily quantified, they may be a valuable imaging biomarker in the field of cerebrovascular disease. We have shown that the MARS scale has generally good intra- and inter-rater reliability for definite CMBs, and hope that it will prove to be a useful contribution to efforts to reliably map brain CMBs. Further studies are needed to establish optimal and standardised MRI protocols for CMB identification; investigate the clinical and diagnostic significance of single (or few) versus multiple CMBs; and develop clear standards for CMB rating. This should allow the combination of reliable data from different centres for effective investigation of the many remaining important clinical questions.

Chapter 5 - Cerebral Microbleeds and Intracerebral Haemorrhage Risk: Exploring the Risk Factors for Intracerebral Haemorrhage in Patients on Anti-platelet Treatment in a Case-Comparison Study

5.1 Summary

ICH is an uncommon but devastating complication of regular antiplatelet use: identifying high risk patients before treatment could potentially reduce this hazard. Brain CMBs on gradient-recalled echo (GRE) T2*-weighted MRI are considered a biomarker for bleeding-prone small vessel diseases. We hypothesised that patients with CMBs are more prone to ICH if they are treated with antiplatelet agents, especially if the CMBs are numerous and lobar. We investigated this in a hospital-based matched case-comparison study. Cases of spontaneous ICH were ascertained using overlapping methods, from a prospective database of 1017 consecutive unselected patients referred to our stroke unit and associated clinics. For each case of antiplatelet-associated ICH, 2 controls matched for age, sex and hypertension without history of ICH on antiplatelet therapy were selected. We found that CMBs were more frequent in antiplatelet users with ICH than in matched antiplatelet users without ICH (13/16[81%] versus 6/32[19%], p=0.004) and patients with non-antiplatelet-associated ICH (13/16[81%] versus 15/33[45%], p=0.03). The frequency of lobar CMBs was 11/16[69%] in antiplatelet-associated ICH versus 11/33[33%] in non-antiplatelet-associated ICH (p=0.032). CMBs were more numerous in antiplatelet users with ICH compared to controls (p=0.016). The number of CMBs was associated with the risk of antiplatelet-associated ICH (adjusted OR 1.33 per additional CMB, 95%CI 1.06-1.66, p=0.013). We concluded that brain CMBs are associated with ICH in patients on anti-platelet treatment. In patients with a large number of lobar CMBs, the risk of ICH could outweigh the benefits of antiplatelet therapy. Due to our small numbers and lack of proven causal association, larger prospective studies to investigate the prognostic significance of CMBs in regular antiplatelet users are warranted.

5.2 Study design

Since CAA is radiologically characterised by CMBs in a lobar distribution in the brain (Vernooij et al., 2008b) we hypothesised that brain CMBs – especially lobar - are associated with antiplatelet-associated ICH. We used a case-comparison study design to compare CMB prevalence and distribution in antiplatelet users with symptomatic ICH compared with matched ICH-free antiplatelet users. Because CMBs are known to be associated with ICH regardless of antiplatelet use, we also conducted a case-case comparison study between antiplatelet users with ICH and patients with spontaneous ICH unrelated to antiplatelets.

5.3 Study methods

5.3.1 Study population

We studied a population of 1017 unselected consecutive patients referred to the Stroke Unit and associated neurovascular clinics at the NHNN from December 2000 to February 2008. The Stroke Unit takes all patients with suspected stroke admitted from the surrounding district and has a policy of performing MRI with gradient-recalled echo (GRE) T2*-weighted sequence in all patients with ICH and IS unless contra-indicated, allowing us to minimise selection bias. MRIs are performed within 5 days of admission or on the day of the clinic. All patients have standard questions including questions about antithrombotic use recorded onto a written proforma.

Cases were patients with spontaneous symptomatic ICH occurring during regular antiplatelet treatment. All patients with ICH were ascertained with overlapping methods including MRI images, reports and medical records. The intake and duration of antiplatelet treatment at the time of admission or visit were ascertained from hospital and GP records. Cases of spontaneous ICH unrelated to antiplatelet agents or other antithrombotic treatment were ascertained from MRI images and medical records. Due to small numbers, all cases of spontaneous ICH unrelated to antiplatelet agents were included and therefore these were not matched with cases of antiplatelet-associated ICH. For each antiplatelet user with ICH two controls matched for age, sex and hypertension and without any history of ICH were selected randomly (blinded to other clinical details or CMB ratings) from prospective databases of consecutive patients admitted to the stroke unit and associated neurovascular clinics. Controls were patients using antiplatelet agents admitted to the department due to suspected IS or TIA.

We recorded the BPs taken at the time of admission or visit. Patients were considered hypertensive when they were on antihypertensive drugs or when their BP was ≥140/90 for more than 7 days after admission. We compared baseline demographics, prevalence of leukoaraiosis and presence, number and distribution of CMBs between cases and controls. We also performed a case-case comparison between antiplatelet users and non-antiplatelet users with ICH.

5.3.2 Standard protocol approval

The study received ethics approval by The NHNN & Institute of Neurology Joint Research Ethics Committee (approval number 07/Q0512/39; date of approval 22 October 2007).

5.3.3 Imaging analysis

MRIs were carried at 1.5T field strength. GRE T2* sequences were obtained in the axial plane on a Genesis Signa Scanner [TR 300 milliseconds, TE 40 milliseconds, FA 20°, FoV 24x18, matrix 256x160, slice thickness 5mm, slice gap 1.5mm, NEX 1]. A minority of patients was scanned on a Siemens Avanto Scanner [TR 800 milliseconds, TE 26 milliseconds, FA 20°, FoV 24x18, matrix 512x448, slice thickness 5mm, slice gap 1.5mm, NEX 1]. CMBs were identified by a neurologist trained in CMB identification (SMG) and blinded to all clinical information, who rated CMB presence, number and distribution on GRE T2* images using MARS, a validated scale for CMB reporting in all brain locations (Gregoire et al., 2009). Leukoaraiosis was defined as the presence of early confluent or confluent WML, corresponding to a Wahlund score equal or above 2 (Wahlund et al., 2001). Lacunes were defined as brain infarcts measuring between 3 and 20mm in size and mostly present in the deep brain structures (Bryan et al., 1999). According to previous descriptions, they were characterised as being hypointense in T1-weighted and FLAIR images, and surrounded by a hyperintense rim in FLAIR images (Kwon et al., 2006; Naka et al., 2006). ICHs were classified as deep, lobar and posterior fossa. According to MARS, CMB distribution was classified as deep, lobar and posterior fossa.

5.3.4 Statistical analysis

Blinded analysis of the data was performed with the Data Analysis and Statistical Software STATA Intercooled version 8.0 and SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL). Conditional logistic regression analysis was used for the comparison of characteristics between the case group (antiplatelet users with ICH) and matched control group (antiplatelet users without ICH). A two-sided independent groups t-test was used for variables that are normally distributed in each group, Fisher's exact test for comparison of proportions, and Mann–Whitney U test for comparison of variables that are not normally distributed in at least one of the two groups. We investigated the effects of the number of CMBs in predicting antiplatelet-associated ICH, with adjustment for leukoaraiosis. The variables entered into the regression analysis model were variables that were significant in

univariate regression. For the case-case comparison study, we used two-sided independent t-test, Fisher's exact tests and non-parametric Mann-Whitney U tests to compare demographics and radiological features between the two groups. Statistical significance was declared if $p<0.05$.

5.4 Results

5.4.1 Demographic results

We identified a total of 164 subjects (N=16%) with ICH admitted during the study period. Of these, we excluded patients with structural and secondary causes of ICH (aneurysms, tumours, cavernomas, arterio-venous malformations, coagulation disorders or use of oral anticoagulants, venous thrombosis or head injuries) (N=69). We then excluded patients in whom the MRI images were not of satisfactory quality (N=3), did not include a GRE T2* sequence (N=8) or were not performed (N=35). Of the remaining 49 patients (30%), we identified 16 cases (patients on regular antiplatelet therapy) and 33 non-antiplatelet users with ICH (Figure 47).

Patients included in the study were slightly older than patients excluded due to a lack of MRI (p=0.043). However, there was no significant difference between the 2 groups for any of the other vascular risk factors: hypertension, systolic BP, diastolic BP, statin use, median cholesterol total, smoking history, diabetes, previous history of stroke, history of AF, ischaemic heart disease (IHD) and antithrombotic use. The proportion of patients scanned on the Siemens Avanto scanner was small: antiplatelet-associated ICH (N=1, 6%), controls (N=2, 6%), non-antiplatelet-associated ICH (N=3, 9%).

We selected 32 matched controls from the database of antiplatelet users without ICH. Cases and controls were well matched for all clinical characteristics and potential confounding factors including previous history of IS, IHD, lacunar infarcts, median dose of aspirin and duration on antiplatelets (Table 12). Antiplatelet agents used by patients and controls were: aspirin (N=43), clopidogrel (N=3), aspirin plus dipyridamole (N=1), or clopidogrel plus aspirin (N=1).

Among patients with ICH related and unrelated to antiplatelet agents, the groups were similar for most clinical characteristics (Table 13), but as expected, previous IHD and stroke were more prevalent among antiplatelet than non-antiplatelet users (p=0.003 and <0.001).

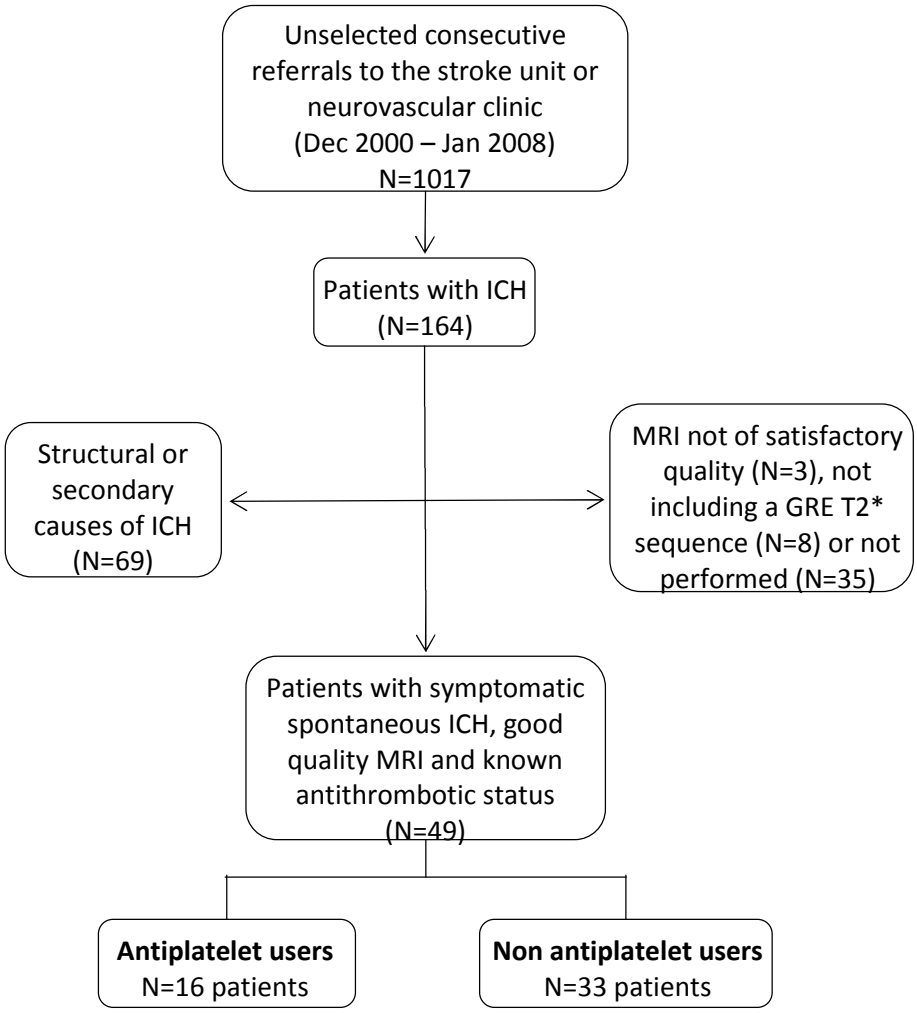


Figure 47. Patient flow diagram of antiplatelet-associated ICH study.

5.4.2 Incidence and location of the intracerebral haemorrhages

The majority of subjects had single ICHs (N=14 [87.5%] and N=28 [85%] in antiplatelet users and non-antiplatelet users). The ICHs were most often located in the cerebral lobes (Table 13). Multiple acute ICHs were seen in 2 antiplatelet users (12%) and 5 non-antiplatelet users (18%). Two patients had prior ICH, one on long-term antiplatelet therapy (6%) and

one non-antiplatelet user (3%). Examples of GRE T2* images of an antiplatelet user who developed two acute lobar haemorrhages are shown in Figure 48.

5.4.3 Comparison between cases and controls

Thirteen (81.2%) antiplatelet users with symptomatic ICH had CMBs compared with six (18.8%) antiplatelet users without any history of ICH (p=0.004) (Table 14). CMBs were more numerous in subjects with ICH compared with controls (median: 6.0, range 0-28, versus median: 0, range 0-15; p=0.016). In both groups, CMBs were most common in the lobes than in the deep or posterior fossa regions. The prevalence of leukoaraiosis was higher in antiplatelet users with ICH compared with controls (50% versus 22%, p=0.069).

Table 13. Characteristics of antiplatelet users with and without history of ICH

	With ICH (N=16)	Without ICH (N=32)	P value
Clinical characteristics†			
Mean age in yrs (range)	70+/-10.5 (52-86)	67.25+/-9.9 (53-94)	*NS*
Men, N (%)	10 (62.5)	20 (62.5)	*NS*
Hypertension (%)	12 (75)	24 (75)	*NS*
Systolic blood pressure (mmHg)	165+/-26	154.85+/-25	*NS*
Diastolic blood pressure (mmHg)	87+/-12	81.5+/-15.51	*NS*
Diabetes (%)	4 (25)	7 (22)	*NS*
Cholesterol total >6mmol/L (%)	1 (6)	3 (9)	*NS*
Statin use (%)	5 (31)	14 (52)	*NS*
Ischaemic heart disease (%)	6 (37.5)	13 (40.6)	*NS*
Atrial fibrillation (%)	2 (12.5)	6 (18.7)	*NS*
Previous stroke (%)	8 (50)	16 (50)	*NS*
Smoking (%)	10 (62.5)	17 (53)	*NS*
Duration, months (%)	41.6+/-48.4	37.7+/-43.4	*NS*
Number taking aspirin (%)	15 (94)	30 (94)	*NS*
Median dose of aspirin in aspirin users in mg (range)	75 (75-300)	75 (75)	*NS*
Median interval stroke-MRI in days (range)	11 (2-115)	17 (0-131)	*NS*
Imaging characteristics			
CMBs			
Presence (%, range)	13 (81.2, 0-28)	6 (18.8, 0-15)	0.004*
Median (range)	6.0 (0-28)	0 (0-15)	0.016*
Lobar			
Presence (%)	11 (69)	5 (15.6)	0.004*
Median (range)	3.0 (0-15)	0 (0-8)	0.016*
Deep (basal ganglia and thalamus)			
Presence (%)	9 (56.2)	3 (9.4)	<0.001*
Median (range)	1.0 (0-11)	0 (0-3)	NS
Posterior fossa (brainstem and cerebellum)			
Presence (%)	6 (37.5)	4 (12.5)	*NS*
Median (range)	0 (0-2)	0 (0-5)	*NS*
Lacunes			
Presence (%)	12 (80)	27 (84)	*NS*
Median (range)	2.0 (0-9)	2.5 (0-10)	*NS*
Leukoaraiosis			
Presence (%)	8 (50)	7 (22)	*NS*

NS: not significant; IQ range: interquartile range
*p<0.05
† Plus–minus values are means+/-SD

Table 14. Characteristics of subjects with symptomatic spontaneous ICH

	Antiplatelet users (N=16)	Non-antiplatelet users (N=33)	P value
Clinical characteristics†			
Mean age in yrs (range)	70+/-10.5 (52-86)	66+/-10.6 (41-87)	*NS*
Men, N (%)	10 (62.5)	18 (54.5)	*NS*
Hypertension (%)	12 (75)	27 (81.8)	*NS*
Systolic blood pressure (mmHg)	165+/-26	151+/-32	*NS*
Diastolic blood pressure (mmHg)	87+/-12	83+/-16	*NS*
Diabetes (%)	4 (25)	2 (6.1)	*NS*
Cholesterol total >6mmol/L (%)	1 (6)	2 (6)	*NS*
Statin use (%)	5 (31)	4 (13)	*NS*
Ischaemic heart disease (%)	6 (37.5)	1 (3)	0.003*
Atrial fibrillation (%)	2 (12.5)	3 (9.1)	*NS*
Previous stroke (%)	8 (50)	2 (6.1)	<0.001*
Smoking (%)	10 (62.5)	10 (30.3)	*NS*
Median interval stroke-MRI in days (range)	11 (2-115)	7 (0-222)	*NS*
Imaging characteristics			
CMBs			
Presence (%, range)	13 (81.2, 0-28)	15 (45.4, 0-31)	0.030*
Median (range)	6.0 (0-28)	0.0 (0-31)	0.012*
Lobar			
Presence (%)	11 (69)	11 (33)	0.032*
Median (range)	3.0 (0-15)	0 (0-12)	0.016*
Deep (basal ganglia and thalamus)			
Presence (%)	9 (56.2)	9 (27.3)	*NS*
Median (range)	1.0 (0-11)	0 (0-17)	0.032*
Posterior fossa (brainstem and cerebellum)			
Presence (%)	6 (37.5)	5 (15)	*NS*
Median (range)	0 (0-2)	0 (0-8)	*NS*
Lacunes			
Presence (%)	12 (80)	24 (75)	*NS*
Median (range)	2.0 (0-9)	2.0 (0-7)	*NS*
Leukoaraiosis			
Presence (%)	8 (50)	8 (24)	*NS*

NS: not significant; IQ range: interquartile range
*p<0.05
† Plus–minus values are means+/-SD

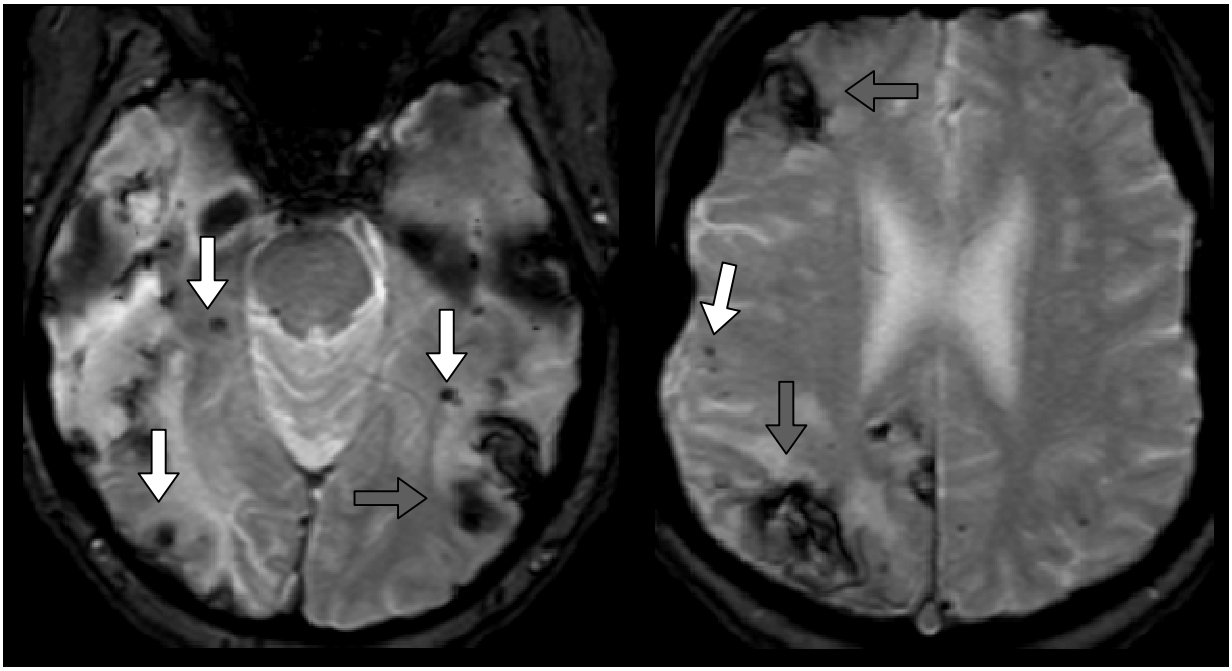


Figure 48. GRE T2* MRI of an antiplatelet user who developed two lobar intracerebral haemorrhages

GRE T2*-weighted MRI of a patient with antiplatelet-associated ICH demonstrating numerous lobar CMBs (white arrows) and three lobar haemorrhages (temporal left, parietal right, frontal right, grey arrows). Cerebral amyloid angiopathy is likely to be the underlying cause of the CMBs and multiple ICHs in this patient.

5.4.4 Factors associated with the risk of ICH in patients on anti-platelet treatment

In a conditional logistic regression, the total number of CMBs was a significant predictor of antiplatelet-associated ICH (OR 1.27, 95% CI 1.04-1.55, p=0.016), even after adjusting for the presence of leukoaraiosis (OR 1.33, 95% CI 1.06-1.66, p=0.013) (Table 14). Lobar CMBs were significantly associated with antiplatelet-associated ICH after adjusting for the presence of leukoaraiosis (OR 1.42, 95% CI 1.07-1.89, p=0.016) whereas deep CMBs were not (OR 5.69, 95% CI 0.95-34.22, p=0.057). However, the OR for the association between antiplatelet-associated ICH and deep CMBs was greater than that of lobar CMBs (albeit with wide CI), and approached statistical significance.

Table 15. Logistic regression analyses testing the factors predicting the likelihood of ICH in antiplatelet users with ICH and antiplatelet users without ICH matched for age, sex and hypertension

	Odds Ratio	95% CI	P value
Number of CMBs			
Total number of CMBs	1.27	1.04-1.55	0.016*
Total number of CMBs adjusted for leukoaraiosis[1]	1.33	1.06-1.66	0.013*
Distribution of CMBs			
Number of Lobar CMBs	1.35	1.06-1.72	0.016*
Number of Lobar CMBs adjusted for leukoaraiosis[2]	1.42	1.07-1.89	0.016*
Number of Deep CMBs	5.86	0.96-35.69	0.055
Number of Deep CMBs adjusted for leukoaraiosis[3]	5.69	0.95-34.22	0.057

*p<0.05

[1]Hosmer-Lemeshow goodness of fit: Chi-Square 15.532, sig. 0.004
Positive predictive value:38 %
Negative predictive value: 77%

[2]Hosmer-Lemeshow goodness of fit: Chi-Square 11.310, sig. 0.023
Positive predictive value: 42%
Negative predictive value: 75%

[3]Hosmer-Lemeshow goodness of fit: Chi-Square 55.535, sig. 0.016
Positive predictive value: 36%
Negative predictive value: 76%

5.4.5 Comparison between cases of intracebral haemorrhage differing in the use of antiplatelets

CMBs were more frequent in antiplatelet users with ICH compared with patients with non-antiplatelet-associated ICH (13/16 [81.2%] versus 15/33 [45.4%]; p=0.030). CMBs were more numerous in antiplatelet users (range 0-28, median 6.0 versus range 0-31, median 0; p=0.012) and lobar CMBs more prevalent (69% versus 33%, p=0.032). Both deep and lobar CMBs were more numerous in antiplatelet users than in non-antiplatelet users (p=0.032 for deep and p=0.016 for lobar CMBs). Finally, leukoaraiosis was more prevalent in antiplatelet users with ICH compared with subjects with ICH unrelated to antiplatelets, but the difference was not statistically significant (50% versus 24%, p=0.106).

5.5 Discussion

In this study we found that CMBs were more prevalent and numerous in antiplatelet users who developed symptomatic ICH compared with matched antiplatelet-users who did not develop ICH. The CMB number was strongly associated with ICH risk even after controlling for the presence of leukoaraiosis and other potential confounding factors. Our study suggests that the association of ICH with CMBs may be more powerful than that with leukoaraiosis, although CMBs and leukoaraiosis may reflect similar pathological damage to small vessels (Naka et al., 2006). In separate regression analyses adjusted for the presence of leukoaraiosis, lobar (but not deep) CMBs were a statistically significant predictor of ICH in patients on anti-platelet treatment. These data support a potential role for brain CMBs, particularly in a lobar distribution, as a risk factor for antiplatelet-associated ICH.

We have confirmed previous observations of a predominance of lobar CMBs in patients with ICH (Roob et al., 2000; Tsushima et al., 2003); we also found that lobar CMBs were more common in antiplatelet-associated ICH than in ICH unrelated to antiplatelet agents ($p=0.032$). Moreover, regression analyses indicated that lobar CMBs were significantly associated with antiplatelet-associated ICH. These observations suggest that lobar CMBs may be more strongly related to antiplatelet-associated bleeding than deep CMBs, although our sample size is too small to confirm this hypothesis, which requires confirmation in larger studies. Previous autopsy and MRI studies suggest that lobar CMBs are related to CAA (Greenberg, 1998; Rosand et al., 2000). In CAA, Aβ-protein is deposited in the walls of superficial cortical and leptomeningeal vessels, causing them to become brittle and prone to bleeding. CAA may account for a large proportion of spontaneous ICH in older people (Wong et al., 2000) and has been suggested as a risk factor for ICH associated with warfarin (Rosand et al., 2000) and aspirin (Wong et al., 2000) as well as with ICH after thrombolysis for IS (McCarron and Nicoll, 2004). These data are consistent with the hypothesis that CAA is an important risk factor for ICH related to antiplatelet use, and suggest that patients with clinical and imaging findings suggestive of CAA should be treated with antiplatelet agents only if there are compelling reasons to treat to reduce their overall risk of ischaemic vascular events.

Very few previous studies have investigated the association between CMBs and antiplatelet-associated haemorrhage (Huang et al., 2008; Wong et al., 2003). Moreover, these were in Chinese patients in whom hypertension and CMBs are more common than in

Europeans, so may not be generalisable to all stroke populations. Nevertheless, Wong et al found CMBs in 19 of 21 aspirin users with symptomatic ICH, compared to 7 of 21 matched aspirin users without ICH, a result consistent with our findings. In contrast with our study, no cases of ICH unrelated to aspirin were included, and no adjustment was made for the presence of leukoaraiosis, a potentially important confounding factor. A recent randomised trial comparing the new antiplatelet drug cilostazol to aspirin found that in the 6 patients who developed ICH, all had previous CMBs in the location of the ICH (Huang et al., 2008); this observation is also consistent with the hypothesis that CMBs could be used to identify patients at the highest risk of antiplatelet-associated ICH, but the small number of outcome events makes it difficult to draw firm conclusions.

Our study has a number of methodological strengths. First, our data were prospectively collected, with standardised clinical data and MRI imaging sequences; because of our policy of performing routine GRE MRI on all patients unless contra-indicated, we were able to minimise selection bias. Second, we matched our cases with our controls, which is a powerful way to identify risk factors for rare outcome events. Given the very low absolute risk of antiplatelet-associated ICH (about 0.1-0.2% per year), prospective studies would require large number of patients and long follow-ups: indeed, the few prospective studies so far available failed to reach definite conclusions because of small sample sizes and low statistical power (Boulanger et al., 2006; Fan et al., 2003; Naka et al., 2006). Third, we were able to specifically investigate the independent effect of CMBs on ICH risk because important known confounding factors (age, sex, hypertension, duration on antiplatelets, and previous history of stroke and presence of leukoaraiosis) were controlled for in the matching process and logistic regression analysis. We adjusted for leukoaraiosis which is a risk factor for warfarin-associated ICH and is associated with CMBs (Algra et al., 1997; Diener et al., 2004; Jeong et al., 2004; Roob et al., 2000; Smith et al., 2002). Fourth, we used a reliable anatomical CMB rating scale with good intra- and inter-observer agreement (Gregoire et al., 2009). Finally, we included patients with ICH unrelated to antiplatelet treatment, ascertained from the same population as our main case group, making it less likely that our finding is confounded by the well-described association of CMBs with ICH overall. However, in this case-case ICH comparison, a greater proportion of patients with ICH taking antiplatelet agents had a history of previous IS, which could contribute to the higher CMB prevalence in this group.

The main limitations of our study include the small cohort and retrospective design. We obtained large CI in the estimation of ICH risk for the number of deep CMBs, likely to be due to the small number of deep CMBs in our cohort overall. We could only include patients who had MRI, which biased the study towards including slightly older and less severe cases of ICH. Our overall prevalence of CMBs might therefore be a bit overestimated. Another limitation comes from the fact that our 'controls' presented with non-ICH stroke symptoms, and therefore were not 'true' controls. However, this should not have biased our study towards positive results as our controls are likely to have more CMBs than 'true' controls. Also, there was a possible lack of comparability between our cases, because these were not matched. Therefore, comparison between their baseline characteristics (e.g. blood pressure) may have failed to reach significance due to small numbers. Finally, like all case-control and case-comparison studies, ours can only provide evidence of an association between CMBs and ICH in patients on anti-platelet treatment, and not that CMBs are causally associated. For these reasons, our results need to be confirmed in other prospective studies.

The study also has a limitation due to the delay of a few days before performing MRI after the ICH, which may lead to survival bias in this condition associated with high early mortality (Leys and Cordonnier, 2010).

5.6 Conclusions

In summary, we have demonstrated an association between CMBs and ICH in patients on antiplatelet treatment. The predominance of lobar CMBs in patients on antiplatelets who develop an ICH suggests that CAA may be an important underlying risk factor for ICH in patients taking antiplatelets. Our findings suggest that patients with numerous CMBs, particularly in a lobar distribution, may be at higher risk of ICH, which could outweigh the benefits of antiplatelet therapy in these patients (Soo et al., 2008). Therefore, CMB screening may be an important component in the design of future antiplatelet trials, especially in primary prevention where the risks of ICH are more likely to outweigh the benefits than in secondary prevention. Large prospective studies and systematic analyses are needed to further clarify the risks and benefits of antiplatelet therapy in patients with CMBs.

Chapter 6 - Exploring Cerebral Microbleed Dynamics in a Case-Control Study

6.1 MRI detection of new cerebral microbleeds in patients with ischaemic stroke

Some recent studies indicate that CMBs have a dose-related effect on the risk of ICH, cognitive dysfunction and mortality (Greenberg et al., 2004a; Greenberg et al., 1999; Werring et al., 2004). Furthermore, the burden of CMBs in the brain may be a useful biomarker to monitor the progression of cerebrovascular disease affecting small vessels; indeed, in CAA, CMBs have been shown to accumulate over time, and to predict recurrent ICH and clinical decline (Greenberg et al., 1999). By contrast, very little is known about whether CMBs develop over time in patients with IS, in whom CMBs are increasingly detected because of the routine use of GRE T2*-weighted MRI in neurovascular clinics (Werring et al., 2005). It has been suggested that the development of CMBs – which cause surrounding tissue damage on histopathological studies – may have important cumulative effects on brain function including cognition (Werring et al., 2004). Thus, CMB development over time, and the factors which may influence this process, is of potentially significant clinical interest in stroke medicine. Although the presence of hypertension, and left ventricular hypertrophy on echocardiography (a marker of hypertension severity and duration) (Henskens et al., 2008) are associated with CMBs in cross-sectional studies, it is not known whether hypertension influences the risk of developing new CMBs over time.

Studies on CMBs using serial MRI largely relate to patients with a symptomatic lobar haemorrhage and probable or possible CAA. Two studies on patients with lobar haemorrhages suspected of CAA showed that 38% to 46% of patients developed new CMBs at follow-ups of 1.5 and 1 year, respectively (Greenberg et al., 1999; Chen et al., 2006). In the first study, the development of new CMBs was related to the number of CMBs at baseline; in the second, it was related to the volume of WML at baseline. In another study, the number of MRI-detected CMBs increased after the onset of ICH in patients with symptomatic spontaneous deep ICH (Imaizumi et al., 2003).

We studied the incidence of new CMBs in a cohort of patients with IS or transient ischaemic attack (TIA) screened for CMBs at baseline. Predictors of new CMBs were tested in logistic regression. At the time of conducting this research, there had been no studies that had investigated how CMBs accumulate over time in patients with IS and the factors influencing this process. It was not known whether vascular risk factors e.g. hypertension or the use of antithrombotic agents influence the rate of accumulation of CMBs. We discuss the rationale for attempting to answer this particular question.

6.2 Study design

We assessed the development of new CMBs in patients with IS and TIA screened for CMBs on GRE T2* MRI at baseline and at 5 year follow-up, with detailed clinical assessments at both time points. We hypothesised that CMBs and higher BP at baseline increase the risk of developing new CMBs.

6.3 Study methods

6.3.1 Study population

Consecutive individuals referred to the neurovascular clinic at the NHNN with suspected IS or TIA from 2001-2002 underwent detailed clinical assessment by a consultant stroke neurologist, and GRE T2* MRI on the same day. On the basis of the results from GRE T2*-weighted MRI, 25 patients with cerebral CMBs and a CMB-free control group (N=30) matched for age, gender and other clinical and radiological characteristics were selected from a previously published study of cognition in patients with CMBs (Werring et al., 2004). Patients with confirmed IS or TIA (N=48) were invited for repeat MRI and clinical assessment. Clinical follow-up assessment included a neurological examination, detailed medical questionnaire, validated disability scales (National Institute of Health Stroke Scale (IHSS), Modified Rankin Scale, Barthel Index) and standardised quality of life measurements (EuroQoL).

6.3.2 Standard protocol approval

The study was approved by the Institute of Neurology and NHNN Joint Research Ethics Committee (approval number 07/Q0512/39; date of approval 22 October 2007). All participants gave written informed consent.

6.3.3 MR imaging and reporting

All baseline and follow-up MRIs were carried out on a GE Medical Genesis Signa 1.5Tesla system using the following imaging protocol: axial T2-weighted FSE (TR 6000, TE 105, FA 90, matrix 256x224, FoV 24x18, slice thickness 5 mm, slice gap 1.5mm, NEX 2); axial GRE T2* (TR 300, TE 40, FA 20, FoV 24x18, matrix 256x160, slice thickness 5mm, slice gap 1.5mm, NEX 1). Similar patient positioning was ensured between studies by a standardised radiographic protocol. Images were assessed by SMG blinded to clinical details using validated rating scales for WML (Wahlund et al., 2001) and CMBs (MARS, Gregoire et al., 2009) in the same conditions as described previously (see chapter 4 and 5). T2-weighted MRI and FLAIR images were used to help exclude common CMB mimics (Gregoire et al., 2009).

6.3.4 Statistical analysis

Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) and the Data Analysis and Statistical Software Stata (Statacorp, 1985) version 10.0. Comparisons between groups at each assessment were carried out using Fisher's exact test for categorical variables, the independent sample T-Test for normally distributed continuous variables, and the Mann-Whitney *U* test for non-normally distributed continuous variables. Univariate and multivariate analyses using binary logistic regression analysis were used to look for the predictors of new CMBs. The variables entered into the multivariate regression analysis model were variables that were significant in univariate regression. We used penalised maximum likelihood regression and likelihood ratio tests to test the mean systolic BP at baseline as a predictor of new CMBs. Significance was declared at $p<0.05$ (two-sided).

6.4 Results

6.4.1 Demographic findings

The overall prevalence of CMBs in patients with IS or TIA in our neurovascular clinic is 30.2%, similar to that of previous studies (Cordonnier et al., 2007). Of the original cohort (N=48: N=21 with CMBs, N=27 CMB-free) 11 patients (23%, N=7 with CMBs [33%]; N=4 CMB-free [15%], p=0.164) died between original study enrolment and follow-up. The only vascular death was a myocardial infarction in the CMB group. Four patients (8%) were lost to follow-up. Of the remaining 33 patients, a final cohort of 21 patients (64%, N=8 with; N=13 without CMBs) underwent repeat MRI and clinical assessment. The two groups were still well matched for clinical characteristics at follow-up (Table 15). The mean interval between assessments was 5.6 years (SD=0.44). All patients were on antihypertensive therapy except two without CMBs.

6.4.2 Imaging findings

At baseline 8 patients had a total of 62 microhaemorrhages (87% lobar, median 4.0, range 1 to 36; Table 15). At follow-up, five patients (23%) had developed 56 new CMBs (all lobar). Among patients with CMBs at baseline, 4 of 8 (50%) developed new CMBs compared with one of 13 (8%) CMB-free patients (p=0.047). Examples of new CMBs are shown in Figure 49. The number of new CMBs in patients with baseline CMBs was significantly higher than in patients without (54 [range per patient 0 to 34, median 1] versus 2 [range per patient 0 to 2, median 0], p=0.025).

Table 16. Baseline patient characteristics

	CMBs (N=8)	CMB-free (N=13)	p
Clinical characteristics			
Female gender (%)	3 (37)	5 (38)	1.000
Median age: years (range)	65 (45-87)	65 (36-76)	1.000
Hypertension (%)	8 (100)	8 (62)	0.111
Median systolic BP: mm Hg (range)	150 (110-165)	135 (120-160)	0.131
Median diastolic BP: mmHg (range)	80 (60-105)	80 (64-94)	0.581
Diabetes (%)	2 (25)	3 (23)	1.000
On antiplatelets (%)	7 (87)	12 (92)	1.000
On anticoagulants (%)	1 (12)	0	0.400
Median time on antiplatelet agents in months (range)	72 (24-108)	78 (48-144)	0.852
On antihypertensive treatment (%)	8 (100)	11 (85)	0.505
History of previous stroke (%)	3 (37)	2 (15)	0.325
Index event: TIA (%)	0	1 (8)	1.000
Presence of lacunar infarcts (%, median, range)	7 (87, 1.5, 0-3)	12 (92, 1.0, 0-3)	0.826
Anterior circulation stroke/TIA (%)	2 (25)	2 (15)	0.618
Posterior circulation stroke/TIA (%)	0	1 (8)	1.000
Imaging characteristics			
White matter changes			
Median total WML score (range)	5 (0-11)	2 (0-5)	0.100

† $p<0.05$
TIA indicates transient ischaemic attack

6.4.3 Predictors of new cerebral microbleeds

The results of the regression analyses are shown in Table 16. CMB presence at baseline predicted the formation of new CMBs at 5 years (OR 12, 95% CI 1.02 to 141.34, p=0.048); the association with the number of CMBs was weak (OR 1.51, 95% CI 0.99 to 2.30, p=0.055). Mean baseline systolic BP predicted new CMBs at follow-up (OR 1.28 per unit increase, 95% CI 1.23 to 1.33, p<0.001) even after adjustment for the presence of CMBs at baseline (OR 1.18, 1.01 to 1.38, p=0.002). All patients who developed new CMBs had a baseline systolic BP >150mmHg. The mean systolic BP at follow-up was higher in patients with new CMBs compared with those without (163+/-20 mmHg versus 141+/-16 mmHg, p=0.020). We found no association between antiplatelet use, presence or number of lacunar infarcts, and new CMBs.

Table 17. Study patients with ≥1 CMB at baseline, follow-up, or both and with corresponding number of CMBs at baseline and follow up and associated clinical events

Patient Number	Frontal			Parieto-occipital			Temporal			Basal ganglia			Infraten-torial			Total			Any clinical event during follow-up
	B	FU	D	B	FU	D	B	FU	D	B	FU	D	B	FU	D	B	FU	D	
1	0	9	+9	1	2	+1	1	7	+6	0	0	=	1	1	=	3	19	+16	None
2	0	0	=	0	0	=	0	0	=	1	1	=	0	0	=	1	1	=	None
3	2	3	+1	0	0	=	1	2	+1	1	1	=	1	1	=	5	7	+2	None
4	5	5	=	1	3	+2	3	3	=	1	1	=	0	0	=	10	12	+2	ICH
5	0	0	=	1	1	=	0	0	=	0	0	=	0	0	=	1	1	=	None
6	0	0	=	3	2	-1	1	1	=	0	0	=	0	0	=	4	3	-1‡	None
7	7	9	+2	20	39	+19	9	16	+7	0	0	=	0	0	=	36	64	+28	None
8	1	1	=	0	0	=	1	1	=	0	0	=	0	0	=	2	2	=	None
9*	0	1	+1	0	1	+1	0	0	=	0	0	=	0	0	=	0	2	+2	None

Light grey columns are values at baseline. Non bolded columns are values at follow-up. Dark grey columns are differences between baseline and follow-up.
(+) indicates no. of additional CMBs at follow-up compared with baseline for the same region of the brain
(-) indicates no. of CMBs less at follow-up compared with baseline for the same region of the brain
*Patient without CMBs at baseline
‡ One parieto-occipital CMB could not be seen on follow-up images because of artefacts in that region
B = baseline; FU = follow-up; D = difference

6.4.4 Clinical follow-up

There were few vascular outcome events among the survivors in this cohort: ICH not at the site of a CMB (N=1, CMB group); non-fatal myocardial infarction (N=1, CMB-free group). The other surviving patients were clinically stable; there was no difference between the 2 groups at follow-up in median Barthel index (100, range 80 to 100 versus 100, range 95 to 100, p=0.190), median total EQ-5D score (80, range 55 to 98 versus 75, range 20 to 90, p=0.228) and median NIHSS (0 in both groups, p=1.000). Median Rankin scores were slightly worse in CMB patients than CMB-free patients at follow up (1, range 1 to 3 versus 1, range 0 to 2, p=0.040).

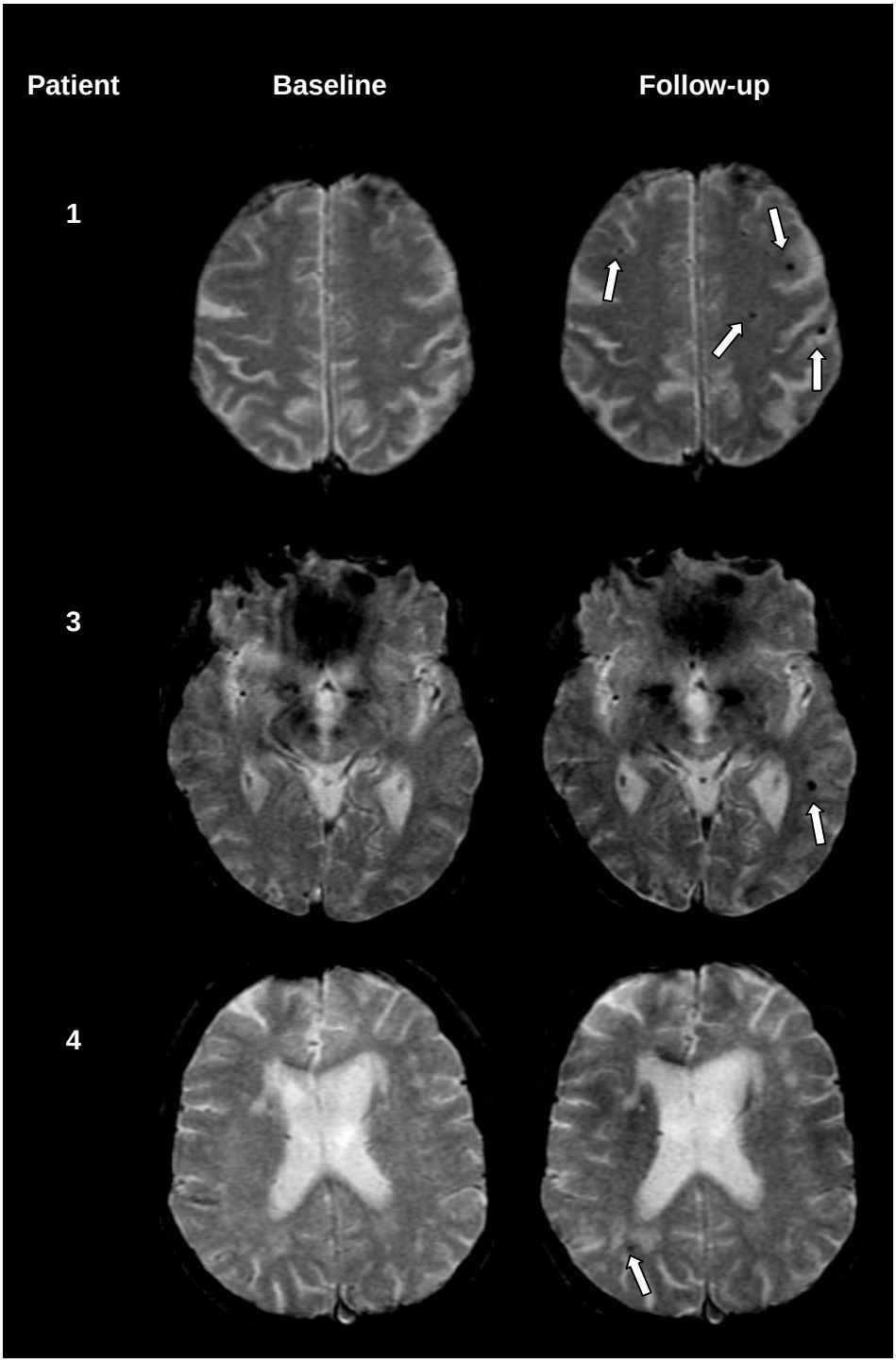


Figure 49. GRE T2*-weighted MRI of patients who developed new CMBs without clinical events over 5-year follow-up.

New CMBs (white arrows) are detected on follow-up images (right panel) but not at baseline (left panel).

Table 18. Univariate and multivariate logistic regression analysis

Factor	Odds Ratio	95% CI	p
Univariate regressions			
Presence of CMBs at baseline	12	1.02-141.34	0.048
Number of CMBs at baseline	1.51	0.99-2.30	0.055
Total score of WML at baseline	1.19	0.83-1.72	0.334
Systolic BP at baseline	1.28	1.23-1.33	<0.001
Multivariate regression*			
Presence of CMBs at baseline	4.27	0.15-122.73	0.396
Systolic BP at baseline	1.18	1.01-1.38	0.002

*Hosmer-Lemeshow goodness of fit: Chi-Square 10.246, sig. 0.175
Positive predictive value: 100%
Negative predictive value: 70%

6.5 Discussion

In this study, 50% of surviving patients with CMBs at baseline developed new CMBs over 5.6 years compared with only 13% of matched CMB-free patients. The presence of CMBs at baseline predicted new CMB development. The risk of developing new CMBs was significantly associated with the mean baseline systolic BP. At the time of conducting this research, no similar data were available in IS cohorts.

We found that baseline systolic BP predicted the development of new CMBs and was above 150 mm Hg in all patients in whom new CMBs developed. Hypertension is an established risk factor for CMBs in cross-sectional studies (Henskens et al., 2008), but we are not aware of previous longitudinal data on whether hypertension increases the risk of developing new CMBs. Therefore, rigorous BP control in patients with IS with CMBs may prevent the progression of small vessel damage and new CMB formation.

Many new CMBs were detected despite patients remaining clinically stable with few clinical events and retention of independence in their daily activities. This suggests that the rate of CMB accumulation may exceed that of clinical vascular events. CMBs in patients with IS may therefore be a more sensitive marker for the progression of small vessel pathology in the brain than clinical outcomes; this could have implications for clinical trials (e.g. investigating the effects of antihypertensive agents) or observational studies.

Previous published data on CMBs using serial MRI largely relates to patients with a symptomatic lobar haemorrhage and probable or possible CAA. A previous study in CAA showed that 38% of patients with lobar haemorrhage developed new CMBs at a follow-up of 1.5 years (Greenberg et al., 1999); the development of new CMBs was related to the number of CMBs at baseline. A subsequent study in CAA patients reported that 46% of patients developed new CMBs after 1 year (Chen et al., 2006); in the latter study, the development of new CMBs was related to the volume of WML at baseline. In our cohort the presence and number of CMBs at baseline (but not the extent of WML) were associated with new CMB formation. Our results support the idea that CMBs in IS patients are a marker for more severe and potentially progressive small vessel pathology, requiring active treatment including careful control of BP and other vascular risk factors.

There is currently no clear evidence to suggest that finding CMBs on MRI should influence the use of antithrombotic agents. There was no association between antiplatelet usage and

new CMB formation in our small study. Recent data suggest that the use of antiplatelet agents may be associated with a higher prevalence of cerebral CMBs in a healthy elderly population (Vernooij et al., 2009) but there are no other data on whether the use of antiplatelet agents increases the rate of accumulation of CMBs over time. We found that antiplatelet-users with large numbers of CMBs may have a substantially higher rate of ICH than those without CMBs, which could cancel out the benefit of these treatments in preventing occlusive vascular events in some patients (Gregoire et al., 2010c) but this hypothesis needs to be tested in other populations (see Chapter 5 for full study details).

CMBs are often considered to be clinically silent; indeed, in our cohort some patients developed many new CMBs despite no apparent clinical deterioration. Nevertheless, CMBs have been associated with neurological symptoms including transient motor or sensory disturbances (Greenberg et al., 1993; Roch et al., 2005) or cognitive dysfunction (Werring et al., 2004). There are very few follow-up studies investigating CMBs and neurological function, but one recent study found that in patients with CADASIL mutations followed up over 7 years, an increased number of CMBs was associated with cognitive decline (Liem et al., 2009). Other studies suggest that CMBs have an independent prognostic significance for mortality or the risk of subsequent ICH in patients with stroke (Soo et al., 2008)or in a memory clinic population (Henneman et al., 2009). Clearly, further follow-up studies are needed to establish how CMBs are related to the development of neurological impairments in stroke patients over time.

Strengths of our study include the use of a long follow-up period, with standardised MRI sequences and a single observer using a validated CMB rating instrument. The main limitation of our study is the small sample size which may affect the generalizability of our findings to the general population of patients with IS, and limits the precision of our estimates of rates of new CMB development. The selection of patients with ischaemic strokes may have excluded asymptomatic patients with potentially larger number of CMBs (e.g. CAA). However, the overall prevalence of CMBs in patients with IS in our neurovascular clinic is 23% (Werring et al., 2005), in line with other estimates (Cordonnier et al., 2007), suggesting that our findings are generalizable. The small sample size also affected the power of our analysis of baseline CMBs as a risk factor for new CMBs. The small p value (p=0.048) suggests that the association found may have not been a real finding and that our results may have been different in a larger population sample. Most CMBs detected at baseline were lobar as were all new CMBs. Although lobar CMBs are

suggested to reflect CAA, whereas deep CMBs are due to hypertensive arteriopathy, the 2 pathologies may interact or coexist (Fazekas et al., 1999). In our study, patients with deep CMBs were overrepresented in patients who died or were lost to follow-up, which may have introduced bias towards detecting new lobar CMBs. We used one-off BP measurements - which are recognised to be of limited value in comparison to more sophisticated techniques including ambulatory BP monitoring, so we could not relate CMB accumulation or clinical events to control of BP during follow-up. Finally, although care was taken to ensure accurate repositioning of patients between MRI studies, it is possible that some of the apparently new CMBs were found because of a change in head position between scans. However, if this was a significant problem we would expect a significant number of 'disappearing' CMBs at follow-up, which was not the case.

6.6 Conclusions

CMBs may be a useful quantitative method for objectively monitoring the progression of small vessel pathology in patients with IS in observational or therapeutic studies (e.g. of antiplatelet or antihypertensive agents). Larger prospective studies are needed to determine whether specific treatments (e.g. aggressive treatment of hypertension) can reduce CMB formation, and how CMB accumulation relates to long-term clinical outcomes.

Chapter 7 - Cerebral Microbleeds and Cognitive Function

7.1 Strictly lobar cerebral microbleeds are associated with executive impairment in patients with ischaemic stroke or TIA

7.1.1 Summary

CMBs are a marker of small vessel diseases, including hypertensive arteriopathy and CAA, and may be associated with cognitive impairment. The relationship between CMBs and cognitive function in ischaemic cerebrovascular disease remains uncertain. We therefore investigated the cognitive impact of CMBs in a cohort of patients with IS or TIA. All patients underwent detailed and comprehensive neuropsychological testing and standardised MRI including FLAIR, T1, T2 and gradient-recalled echo T2*-weighted sequences. CMBs, WML, lacunes and territorial cortical infarcts (defined by standardised criteria) were identified, and associations with cognition assessed. Three hundred and twenty patients with a diagnosis of IS or TIA were included. Of these, 72 (22.5%) had at least one CMB. Of all the cognitive domains tested, only executive impairment was more prevalent in patients with CMBs than without (38% versus 25%; *p*=0.039). In univariate analysis, the presence of strictly lobar (but not deep) CMBs was associated with executive impairment (OR 2.49, 95%CI 1.16-5.36, p=0.019). In adjusted multivariate analyses, the presence (OR 2.34, 95%CI 1.08-5.09, p=0.031) and number (OR1.33, 95%CI 1.04-1.69, p=0.022) of strictly lobar CMBs were significantly associated with executive impairment. CMBs were not associated with impairment in other cognitive domains. In conclusion, strictly lobar CMBs are independently associated with executive dysfunction in patients with IS or TIA. Our findings suggest that a microangiopathy related to strictly lobar CMBs (e.g. CAA) contributes to cognitive impairment in this population.

7.1.2 Rationale

Cognitive impairment after stroke is associated with significant functional impact and high risk of dementia (Wentzel et al., 2001), which might be preventable with improved understanding of the underlying mechanisms. Cerebral small vessel disease plays a key role in cognitive dysfunction (Anon, 2012). CMBs, detected on gradient-recalled echo (GRE) T2*-

weighted MRI, are an important marker of small vessel pathology; they correspond to small perivascular haemosiderin deposits related to vessels affected by hypertensive arteriopathy and CAA, and are presumed to represent focal areas of previous bleeding (Fazekas et al., 1999). Recent evidence suggests that CMBs are linked to cognitive impairment (Cordonnier et al., 2006; Goos et al., 2009), particularly executive function or processing speed (Seo et al., 2007; Werring et al., 2004; Yakushiji et al., 2008). There is indirect evidence that CMB location reflects the underlying small vessel disease process (strictly lobar CMBs being a marker for CAA; deep CMBs for hypertensive arteriopathy) (Poels et al., 2010), but the importance of CMB location with regard to cognition remains uncertain.

Some previous studies of CMBs and cognition have been limited by not considering a full spectrum of cerebrovascular lesions (Fisher, 2010) or using insensitive cognitive screening instruments. The role of CMBs in ischaemic cerebrovascular disease without dementia remains largely unexplored. We therefore investigated the association of CMBs with detailed neuropsychological measures of cognition, taking account of other MRI markers of cerebrovascular disease, in a hospital-based IS or TIA population. We hypothesised, based on observations in our previous preliminary report (Werring et al., 2004) that CMBs are independently associated with domain-specific executive impairment in a larger cohort of patients with IS or TIA, and investigated whether lobar or deep CMBs were most strongly associated with cognitive dysfunction.

7.1.3 Methods

Patients

Patients referred to the stroke service at the NHNN, London, UK, and referred for detailed cognitive testing were considered. During the study period the NHNN received all stroke referrals from the surrounding healthcare districts. Neuropsychological testing is performed routinely in all patients with adequate English, unless they are too unwell (e.g. reduced conscious level) or have severe cognitive impairment (e.g. severe dysphasia or dementia). Inclusion criteria were: standardised adequate quality MRI, including GRE T2* T2-weighted and Fluid Attenuation Inversion Recovery (FLAIR); and complete neuropsychological data (including at least one measure of both executive function and speed and attention processing) within 3 months of the MRI scan. Exclusion criteria included: previous traumatic brain injury or ICH; or known cause of cognitive impairment

other than cerebrovascular disease. All patients had detailed standardised clinical diagnostic assessment including neurological examination, BP measurement, ECG and echocardiogram. We ascertained stroke mechanism using the Trial of Org 10172 in Acute Stroke (TOAST) criteria (Adams et al., 1993). The study was approved by the NHNN and Institute of Neurology joint research ethics committee (approval number 07/Q0512/39; date of approval 22 October 2007).

Imaging

All patients had MR imaging according to a standardised protocol on a 1.5 Tesla scanner: Signa Echospeed (General Electric, Milwaukee, WI, USA), including the following sequences: sagittal T1; axial T2-weighted FSE; axial T2*-weighted GRE (TR 300ms, TE 40ms, FA 20, FoV 24x18, matrix 256x160); and coronal FLAIR (TR 9895ms, TI 2473ms, TE 140ms). For all these sequences, slice thickness was 5 mm and slice gap 1.5 mm. Image analysis for CMBs was conducted by a neurologist trained in neuroimaging (SMG) blinded to clinical details. CMBs were categorised as lobar, deep or infratentorial using the MARS (Gregoire et al., 2009), an instrument with good intrarater and interrater reliability for the presence of definite CMBs in all brain locations using different MRI sequences and observer experience (Gregoire et al., 2009). CMBs in the insula were classified as lobar. WML were rated on axial T2-weighted and coronal FLAIR images using a validated scale (Wahlund et al., 2001) by SMG and another trained observer (GS). Interobserver agreement for WML showed intraclass correlations between 0.84-0.96. Images were assessed for the number of lacunes, defined as CSF-containing spaces, between 2-20 mm diameter, with high signal on T2-weighted or FLAIR images, or with a perilesional halo on FLAIR images (Vermeer et al., 2007). Territorial cortical infarcts were defined as involving the cortical territory of a large cerebral artery (not suggesting a perforating vessel occlusion), with a diameter above 15 mm.

Neuropsychological data

Neuropsychological assessment (blinded to CMB status) comprised a standardised battery evaluating 7 cognitive domains: current intellectual functioning, speed and attention, verbal and visual memory, naming skills, perceptual functions, speed and attention and executive functions. Executive functions were examined using two or more of the following: Stroop test (Trennary et al., 1989), Word fluency (Spreen and Benton, 1969), Trail Making Test Part B (U.S. Army, 1944), Weigl Colour Form Sorting Task (Weigl, 1941), and Modified Card Sorting Test (Nelson, 1976). The tests used to evaluate the other

cognitive domains are described in detail elsewhere (Werring et al., 2004). Derived scores were calculated for each test based on published normative data from a sample of individuals of comparable age. Each patient's performance in each cognitive domain was classified as impaired or unimpaired, according to predefined criteria (Werring et al., 2004).

Statistical analysis

Statistical analysis was performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA). The characteristics of patients with and without CMBs were compared using two-sided independent t-test for normally distributed variables, Fisher's exact test for comparison of proportions when the expected number of counts was less than 5 (otherwise Chi-Square test was used), and Mann–Whitney U test for comparison of non-normally distributed variables. Factors associated with executive impairment were investigated in unadjusted logistic regression analysis. Significant variables were subsequently entered into multivariate binary logistic regression analysis. Other variables entered into the multivariate regression models were variables potentially playing a significant role as confounders in the association tested. Significance was declared at $p<0.05$.

7.1.4 Main findings

Of 1335 patients considered, 320 patients with a final diagnosis of IS or TIA were included (Table 18; Figure 50). Of the 320 patients, 254 (79%) had an IS and 66 (21%) had a TIA. At least one CMB was identified in 72 patients (22.5%). The CMB group included a higher proportion of men, a higher prevalence of hypertension, diabetes, antithrombotic use and previous IS or TIA, a greater number of lacunes, and more severe WML compared to the group without CMBs.

Table 19. Characteristics of the patients

	Patients with CMBs (N=72)	Patients without CMBs (N=248)	*p*
General characteristics			
Gender: females (%)	20 (28)	110 (44)	0.012*
Age in years, mean (SD)	67 (12)	63 (15)	0.091
Pre-morbid IQ score, median (range)	98 (50-124)	97 (56-131)	0.843
Number years of education, median (range)	10 (5-18)	10 (0-20)	0.844
Hypertension (%)	63 (88)	175 (72)	0.008†
Systolic blood pressure, mean in mmHg (SD)	149 (23)	146 (26)	0.404
Diastolic blood pressure, mean in mmHg (SD)	84 (13)	82 (14)	0.494
Diabetes (%)	23 (32)	40 (16)	0.004†
Previous ischaemic stroke/TIA (%)	28 (40)	63 (26)	0.033*
Antithrombotic use (%)	36 (50)	89 (37)	0.044*
Clinical classification			
Stroke diagnosis (%)	59 (82)	195 (79)	0.540
Anterior circulation stroke/TIA (%)	52 (74)	184 (78)	0.481
Posterior circulation stroke/TIA (%)	18 (26)	50 (21)	0.434
TIA diagnosis (%)	13 (18)	53 (21)	0.540
Ischaemic stroke/TIA mechanism			
Atherothrombotic (%)	12 (17)	42 (18)	0.859
Cardioembolic (%)	7 (10)	48 (20)	0.043*
Small-artery occlusion (%)	34 (47)	81 (34)	0.040*
Undetermined (%)	16 (22)	52 (22)	0.933
Other determined cause (%)	3 (4)	16 (7)	0.580
Imaging classification			
Median number of lacunes (range)	1 (0-6)	0 (0-3)	<0.001†
Presence of territorial cortical infarct (%)	26 (36)	117 (47)	0.096
Median N cortical infarcts (range)	0 (0-3)	0 (0-5)	0.196
Large Vessel Infarct			
Anterior circulation: ACA (%)	3 (4)	2 (0.8)	0.083
Anterior circulation: MCA (%)	8 (11)	33 (14)	0.546
Bilateral (%)	5 (7)	17 (7)	0.995
Median WML Scores			
Total (range)	10 (0-22)	7 (0-26)	<0.001†

IQ = intelligence quotient; ACA = anterior cerebral artery; MCA = middle cerebral artery; SD = standard deviation; TIA = transient ischaemic attack; WML = white matter lesions.
*$p < 0.05$; †$p < 0.01$

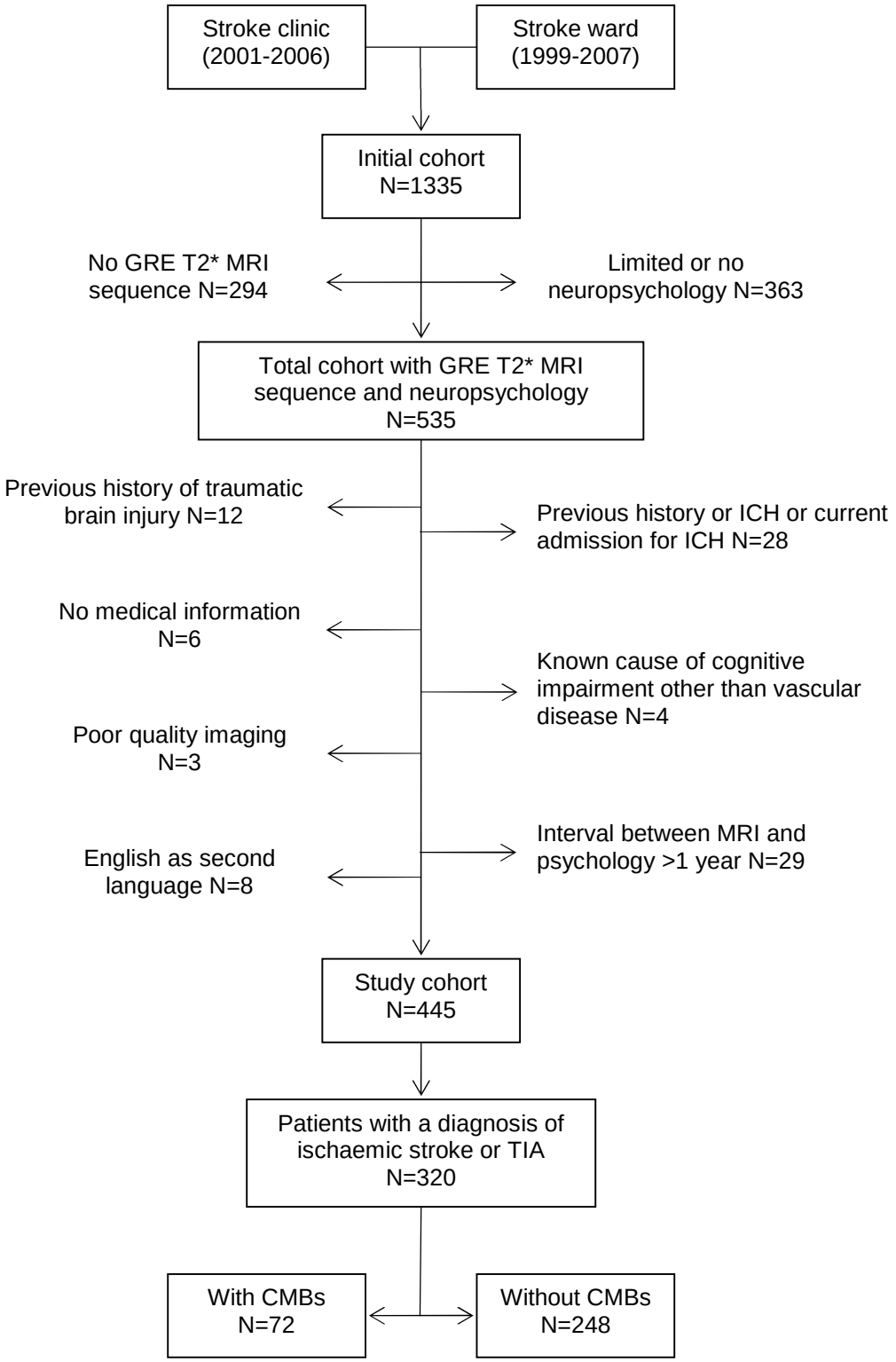


Figure 50. Flowchart describing patient selection for study 7.1

Lobar CMBs were found in 51 patients (71%), deep CMBs in 37 (51%) and infratentorial CMBs in 21 (29%) patients. Lobar CMBs were most common in the temporal (N=31, 61%) followed by parieto-occipital (N=27, 53%) and frontal lobes (N=24, 47%). Thirty patients (42%) had strictly lobar CMBs; 16 (22%) had strictly deep CMBs. Among patients with strictly lobar CMBs, 6 had ≥5 CMBs. Of the domains tested, only executive impairment was more prevalent in patients with CMBs compared to those without (38% versus 25%, p=0.039). There was no significant difference (P>0.10) between the CMB and non-CMB groups in the proportion impaired in the other cognitive domains (Table 19).

Table 20. Proportions of patients impaired in each cognitive domain

Cognitive domains	Patients with CMBs (N=72)	Patients without CMBs (N=248)	*p*
Global cognitive (%)	9 (22)	50 (32)	0.209
Verbal memory (%)	8 (11)	28 (12)	0.894
Visual memory (%)	11 (16)	58 (24)	0.161
Naming skills (%)	8 (11)	28 (12)	0.894
Perceptual function (%)	13 (18)	38 (15)	0.586
Executive function (%)	27 (38)	62 (25)	0.039*
Speed and attention (%)	39 (63)	129 (59)	0.624
Median number of domains impaired (range)	2 (0-5)	1 (0-7)	0.403

Chi-Square test (2-sided) used to test each domain; Mann Whitney U test for mean number of domains.
*p<0.05

In unadjusted binary logistic regression, executive impairment was associated with the presence of ≥1 strictly lobar CMBs and with ≥5 strictly lobar CMBs (Table 20). The number of lacunes and mean WML severity were not associated with executive impairment.

Table 21. Unadjusted univariate binary logistic regression analyses testing the factors associated with executive impairment

Factors	Odds ratio	95% CI	*p*
Age	1.01	0.99-1.02	0.456
Gender (male)	1.02	0.62-1.68	0.945
Hypertension	1.51	0.83-2.78	0.180
Diabetes	1.12	0.61-2.04	0.721
Number of lacunes	1.13	0.82-1.56	0.440
Antithrombotic use	1.06	0.64-1.75	0.826
Previous stroke/TIA	1.29	0.76-2.21	0.334
CMBs			
Overall presence	1.79	1.03-3.13	0.040*
≥ 1 lobar CMB	1.86	0.99-3.48	0.052
≥ 1 deep CMB	0.95	0.44-2.06	0.900
≥ 1 strictly lobar CMBs	2.49	1.16-5.36	0.019*
≥ 5 lobar CMBs	13.63	1.57-118.38	0.018*
WML			
Mean total	1.02	0.97-1.07	0.535

*$p < 0.05$

In multivariate analysis adjusted for age, WML severity and hypertension, the presence of ≥1 strictly lobar CMB and of ≥5 strictly lobar CMBs remained significantly associated with executive impairment (≥1: OR 2.34, 95%CI 1.08-5.09, p=0.031; ≥5: OR 13.72, 95%CI 1.55-121.57, p=0.019) (Table 21). The number of strictly lobar CMBs was associated with an increased likelihood of executive impairment (OR 1.33 per additional CMB). CMB presence in the parieto-occipital lobes (adjusted OR 3.05, 95%CI 1.33-6.96, p=0.008) and insula (adjusted OR 5.55, 95%CI 1.58-19.53, p=0.008) was associated with executive impairment; the presence of infratentorial CMBs showed a non-significant trend towards association with executive function (OR 2.39, 95%CI 0.94-6.05, p=0.067). In other brain regions, CMBs presence was not associated with executive impairment.

Table 22. Multivariate binary logistic regression analyses testing the factors associated with executive impairment

Predictors	Odds ratio	95% Confidence interval	*p*
MODEL 1			
Presence of strictly lobar CMBs (versus none)	2.34	1.08-5.09	0.031*
Age	1.00	0.98-1.02	0.743
Hypertension	1.38	0.69-2.74	0.357
Mean total score of WML	0.99	0.94-1.05	0.818
MODEL 2			
Presence of ≥5 strictly lobar CMBs (versus none)	13.72	1.55-121.57	0.019*
Age	1.00	0.98-1.02	0.998
Hypertension	1.56	0.78-3.11	0.206
Mean total score of WML	0.99	0.93-1.05	0.715
MODEL 3			
Number of strictly lobar CMBs	1.33	1.04-1.69	0.022*
Age	1.00	0.98-1.02	0.822
Hypertension	1.45	0.73-2.88	0.284
Mean total score of WML	0.99	0.93-1.05	0.647

*$p < 0.05$

MODEL 1

*Hosmer-Lemeshow goodness of fit: Chi-Square 9.147, sig. 0.330
Positive predictive value: 60%
Negative predictive value: 72%

MODEL 2

*Hosmer-Lemeshow goodness of fit: Chi-Square 7.532, sig. 0.481
Positive predictive value: 17%
Negative predictive value: 73%

MODEL 3

*Hosmer-Lemeshow goodness of fit: Chi-Square 10.084, sig. 0.259
Positive predictive value: 50%
Negative predictive value: 72%

7.1.5 Discussion

In patients with IS or TIA, CMBs were associated with impairment in executive functions, but not other cognitive domains. Strictly lobar (but not deep) CMBs were associated with executive impairment, independent of age, hypertension and the severity of WML. The presence of at least one strictly lobar CMB more than doubled the likelihood of executive impairment, with evidence of a graded increase in the risk of impairment with an increasing number of strictly lobar CMBs.

CMBs have been reported to be common in various populations. However, links between CMBs and cognitive function have been inconsistent, probably because of the great variability in populations, MRI techniques and cognitive rating instruments used (Cordonnier et al., 2006; Goos et al., 2009; Poels et al., 2012a; Qiu et al., 2010; Seo et al., 2007; van Norden et al., 2011; Werring et al., 2010; Yakushiji et al., 2008). Nevertheless, consistent with our findings, a large recent study in community-based elderly subjects showed that CMBs were independently associated with lower scores in processing speed and executive functions (Qiu et al., 2010). Another study in a large European population-based cohort (Poels et al., 2012a) reported robust associations between strictly lobar CMBs and cognitive function (information processing speed and motor speed), but only weak associations for deep or infratentorial CMBs.

The association between CMBs and cognitive function in stroke cohorts (Werring et al., 2005) is largely unknown. In a small case-control study of 55 patients referred to a neurovascular clinic, we found that executive impairment was twice as common in patients with CMBs as in matched CMB free controls, independent of WML (Werring et al., 2004). These effects were hypothesised to be mediated by CMBs in frontal and basal ganglia regions. In the current study, executive dysfunction was associated only with strictly lobar CMBs, with no significant association with deep or infratentorial CMBs. The different findings in the present study may be explained by the inclusion of only outpatients with less severe cerebrovascular disease and mainly deep CMBs in the previous report (Werring et al., 2004). A study in patients with neuroimaging evidence of small vessel disease reported that frontal and temporal lobe CMBs were related to global cognitive function, psychomotor speed, and attention (van Norden et al., 2011). Finally, a hospital-based study of 411 patients with IS, haemorrhagic stroke and non-stroke (Yamada et al., 2012) found that ‘dementia’ was associated with CMBs in the IS subgroup, but the study did not report adjusted analyses, nor the criteria used to define dementia.

Our study provides evidence for a link between a process associated with strictly lobar CMBs and executive function in ischaemic cerebrovascular disease. Strictly lobar CMBs are a putative marker for CAA (Vernooij et al., 2008b), making this a possible risk factor for cognitive impairment, even in patients in whom CAA was clinically not suspected. Our finding of frequent CMBs in the temporal and parieto-occipital regions is in keeping with the anatomical predilection of CAA (van Norden et al., 2011; Vernooij et al., 2008b). CAA, although most often clinically recognised as a cause of lobar ICH in the elderly, is also

associated with dementia (Charidimou et al., 2012b). CAA pathology is more prevalent at autopsy in the brains of individuals who were previously demented than those who were not demented (Pfeifer et al., 2002). The severity of CAA may also increase the risk of future cognitive decline: in a prospective cohort study of patients with lobar ICH, the risk of cognitive impairment at 2 years increased with increasing baseline CMB burden (hazard ratio [HR] 1.9, 95% CI 1.2 to 2.8, for each increase in CMB category) (Greenberg et al., 2004b). Community-based pathological studies including the Honolulu-Asia Aging Study (HAAS) and MRC Cognitive Function and Aging Study (CFAS) found CAA to be associated with cognition even after controlling for age and neurodegenerative pathology (Pfeifer et al., 2002; Wharton et al., 2011). The Religious Orders study showed that moderate-to-severe CAA was linked to impairment in specific cognitive domains, particularly 'perceptual speed', assessed with the Symbol Digit Modalities Tests and Number Comparison tests (Arvanitakis et al., 2011); however, we found no association between CMBs and 'speed and attention' functions, also assessed by the Symbol Digit Modalities test. Possible explanations include the following: first, our patients all attended hospital with IS or TIA, while the Religious Orders study included community-based subjects, whom only 36% had cerebral infarction at autopsy; and second, the Religious Orders Study found associations with moderate-to-severe CAA pathology, whereas our patients with strictly lobar CMBs probably have less severe CAA pathology. Indeed, without pathological data, we do not know the true prevalence or severity of definite CAA pathology. Nevertheless, we suggest that our data add to emerging evidence that CAA has an independent association with cognitive function in a range of populations.

It is unclear whether CMBs are a direct cause of cognitive impairment or are a general marker for the severity of microangiopathic disease (Werring et al., 2010); our finding that CMBs in the parieto-occipital lobes and insula were associated with executive impairment most favours an indirect link. Mechanisms by which lobar CMBs (reflecting CAA) could be linked with cognitive impairment include vascular Aβ damaging the neurovascular unit (Iadecola C et al, Stroke 2009), hypoperfusion from small vessel stenosis, or even small areas of 'microinfarction' (Gregoire et al., 2011). Some CMBs may have a direct effect on surrounding or connected brain regions, a notion supported by tissue necrosis seen in histopathological studies (Fazekas et al., 1999; Schrag et al., 2010). Precise mapping of CMBs throughout the brain, with assessment of damage to surrounding tissues and the whole brain using quantitative MRI techniques (e.g. diffusion tensor imaging) may be a helpful future approach (van Norden et al., 2011).

The pattern of CMBs we observed (prevalent in the temporal, parieto-occipital and frontal lobes) has previously been associated with cognitive dysfunction in different populations (Pettersen et al., 2008; Seo et al., 2007). A recent study of non-demented elderly subjects also noted that frontal and temporal CMBs were associated with poor cognitive performance (van Norden et al., 2011) although this cohort was restricted to patients with small vessel disease, with a much lower prevalence of cortical infarcts than ours.

Strengths of our study include: the standardised, detailed neuropsychological battery; the standardised MRI protocol tailored to the detection of cerebrovascular disease; and the use of validated rating scales by trained raters blinded to clinical details. We considered a range of cerebrovascular lesions (lacunes, WML, cortical infarcts), and adjusted analyses for potential confounding factors. Despite this, we cannot exclude an effect of other unmeasured confounders (e.g. cerebral volume loss). There may have been selection bias because of our inclusion criteria: patients with very severe stroke who died soon afterwards will not have had MRI or neuropsychology, and patients with severe dementia are unlikely to have been referred to our outpatient service. However, the routine use of neuropsychology means that our results are likely to be generalisable to other stroke service cohorts of stroke survivors able to undergo neuropsychological testing. The use of a routine clinical MRI without optimised sequence parameters or advanced post-processing may have underestimated the prevalence of CMBs, although such technical factors do not seem to markedly affect the relationship between CMBs and cognition (Goos et al., 2011). Nevertheless, it remains possible that highly optimised sequences (e.g. high field strength) could affect the relationship between CMBs and cognition (Conijn et al., 2011). Finally, we were not able to definitely confirm that strictly lobar CMBs were correlated with histopathological or in vivo amyloid imaging evidence of CAA.

7.1.6 Conclusion

Our findings suggest that strictly lobar CMBs may be an independent risk factor for executive impairment in patients with ischaemic cerebrovascular disease. They support the hypothesis that a microangiopathy related to strictly lobar CMBs (e.g. CAA) contributes to cognitive impairment in this population. With increasing knowledge of the pathophysiology and clinical-radiological spectrum of CAA (Charidimou et al., 2012b), our findings may have implications for the diagnosis, treatment and prevention of cognitive impairment in stroke populations.

7.2 Cerebral microbleeds and long-term cognitive outcome: longitudinal cohort study of stroke clinic patients

7.2.1 Summary

Cognitive impairment causes significant disability in the elderly, and is common following IS. Although the underlying mechanisms and prognostic factors remain unclear, small vessel diseases are known to contribute. CMBs are a MRI manifestation of small vessel diseases and may contribute to VCI, particularly executive functions. We hypothesised that baseline CMBs would predict long-term cognitive outcome, specifically executive function. A cohort of consecutive patients found to have CMBs when first referred to a stroke clinic, together with a CMB-free control group matched for age, gender and clinico-radiological characteristics, were invited for follow-up cognitive assessment a median of 5.7 years later. MRI and detailed cognitive assessment (including current intellectual function, verbal memory, visual memory, naming skills, perceptual functions, executive function and speed and attention) were performed at baseline and follow-up. Patients were classified (blinded to MRI and clinical data) as impaired or unimpaired in each domain using pre-defined criteria. We compared the prevalence of cognitive impairments in each domain at baseline and follow-up and investigated clinical and radiological predictors (including baseline CMBs and WML) of executive cognitive impairment.

Of the original cohort of 55 patients, 13 died without follow-up. Twenty-six of the surviving patients (9 with, 17 without baseline CMBs) agreed to follow-up neuropsychological assessment; 21 of these patients had a repeat MRI scan. The median number of cognitive domains impaired increased, regardless of the presence of baseline CMBs (with baseline CMBs: median = 3 [0-5] at follow-up vs median 2 [0-2] at baseline, p=0.016; without CMBs: median 1.0 [0-5] at follow-up vs median 0 [0-5] at baseline, p=0.035). Frontal-executive impairment at follow-up was more prevalent in patients with baseline CMBs than without (78% vs 29%, p=0.038). The presence of baseline CMBs predicted executive impairment at follow-up (OR 8.40, 95%CI 1.27-55.39, p=0.027). Fifty percent of patients with CMBs versus 8% of patients without baseline CMBs developed new CMBs (p=0.047). The severity of WML increased; the difference was statistically significant only in patients without baseline CMBs (p=0.027). There were no new cortical infarcts.

The conclusion is that in stroke clinic patients, CMBs are consistently associated with executive impairment; baseline CMBs are associated with executive impairment at follow-up after 5.7 years. The presence of CMBs has prognostic relevance for long-term cognitive outcome in stroke clinic patients, and may help to optimally target preventive strategies in individuals at highest risk of cognitive decline.

7.2.2 Rationale

VCI causes significant disability in the elderly, and is a common outcome following IS. Although the underlying mechanisms and prognostic factors remain unclear (Bowler, 2005), small vessel diseases including hypertensive arteriopathy or amyloid angiopathy are known to contribute (Schneider, 2007). Optimising control of vascular risk factors may prevent the progression of cognitive impairment (Purandare, 2009), but these strategies require the identification of individuals most at risk of deterioration. To identify individuals at particular risk for cognitive decline, it is critical to understand the mechanisms and prognostic factors for long term cognitive impairment after stroke.

CMBs are an imaging biomarker of small vessel pathology with potential relevance for cognitive outcome (Seo et al., 2007): some studies suggest an association between CMBs and executive function (Liem et al., 2009; Qiu et al., 2010; Werring et al., 2004), yet their prognostic relevance for long-term cognitive outcome in stroke patients has not been investigated. Indeed, very few studies have explored the detailed profile of cognitive impairment in different cognitive domains in stroke survivors: although executive dysfunction is a common early feature (Ballard et al., 2002), few data are available on longer term cognitive dysfunction. In this study, we undertook detailed neuropsychological assessments to test the hypothesis that baseline CMBs (a marker of small vessel pathology) are associated with executive impairment after 5.7 years of follow-up in a cohort of stroke clinic patients.

7.2.3 Study methods

Consecutive patients referred to a stroke clinic (at the NHNN Queen Square, London) found to have CMBs were matched to a non-CMB control group for clinico-radiological characteristics when they first attended the clinic (2001-2002), as previously described (Werring et al., 2004). These patients were invited to attend a follow-up examination as part of this research (2007-2008). All patients had a detailed neuropsychological

assessment and MRI (unless contraindicated) at baseline and follow-up (Werring et al., 2004). The initial cohort consisted of 55 patients (N=25 with CMBs, N=30 without CMBs). Baseline and follow-up MRIs were assessed for CMBs and WML using validated rating scales (Gregoire et al., 2009; Wahlund et al., 2001), and for cortical infarcts, by a trained observer (SMG) blinded to clinical details. A standardised comprehensive neuropsychological test battery was used at baseline and follow-up [see reference 5 for a detailed description], and included assessments of cognitive function in 7 domains: current intellectual functions, verbal memory, visual memory, naming skills, perceptual functions, executive functions; and speed and attention. All these areas of cognition were evaluated using standard neuropsychological tests. Executive functions were examined using two or more of the following tests: Stroop Test (Trennary et al., 1989); Word fluency (Spreen and Benton, 1969), Trail Making Test Part B (U.S. Army, 1944), Weigl Colour Form Sorting Task (Weigl, 1941) and Modified Card Sorting Test (Nelson, 1976). The neuropsychologist was blinded to clinical details and MRI data at the time of assessing the patients. Patients were classified (blinded to MRI and clinical data) as impaired or unimpaired in each domain using standardised pre-defined criteria as described elsewhere (Werring et al., 2004). We recorded cortical territorial infarcts, CMBs, and WML, at baseline and at follow-up. Statistical analysis was performed using SPSS version 17 (SPSS Inc., Chicago, IL, USA). The study was approved by our research ethics committee (approval number 07/Q0512/39; date of approval 22 October 2007). All patients provided written informed consent.

7.2.4 Results

Thirteen out of the original 55 patients died between the baseline examination and the follow-up study. Of the 42 surviving patients, 9 (6 male) with baseline CMBs and 17 patients without (10 male) consented to follow-up (Figure 51). The median time between assessments was 5.67 years (4.75-7.08) (Table 22). Baseline CMBs were present in lobar regions in 8/9 (89%) and in deep regions in 3/9 (33%) patients.

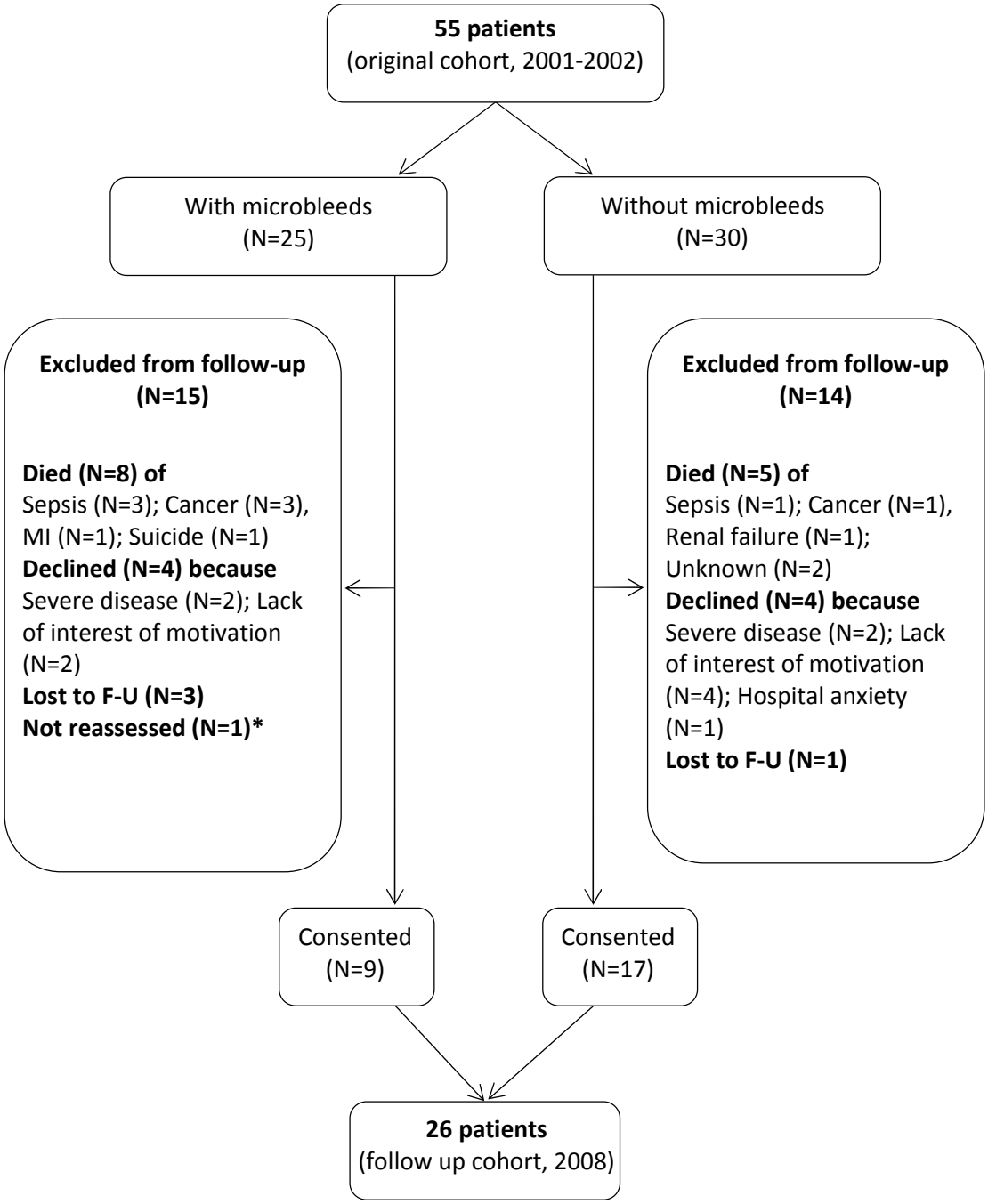


Figure 51. Flowchart describing patient selection for study 7.2

Table 23. Baseline characteristics of CMB and CMB-free patient groups in the 5.7 year follow-up cohort

	With CMBs (N=9)	Without CMBs (N=17)	p
Demographics			
Male gender (%)	6 (67)	10 (59)	1
Median age at first assessment (range)	65 (44-86)	62 (35-75)	0.560
Median education in years (range)	13 (9-24)	11 (0-20)	0.230
Median NART score (range)	95 (73-116)	105 (86-126)	0.132
Median interval between assessments in years (range)	5.67 (4.75-6)	5.67 (5.17-7.08)	0.244
Hypertension (%)	8 (89)	9 (53)	0.087
Median systolic BP (range)	150 (120-160)	135 (110-165)	0.100
Median diastolic BP (range)	80 (60-105)	80 (64-94)	0.738
Smoking (%)	3 (33)	8 (47)	0.683
Median cholesterol in mmol/L (range)	5 (3.8-6.8)	6 (1-8.3)	0.324
Diabetes (%)	2 (22)	4 (23)	1
Previous history of stroke (%)	3 (33)	3 (18)	0.628
Diagnosis			
Ischaemic stroke (%)	9 (100)	13 (77)	0.263
Lacunar (%)	7 (78)	13 (77)	1
TIA (%)	0	1 (6)	1
Non stroke diagnosis (%)	0	2 (12)	0.529
Anterior stroke (%)	2 (22)	2 (12)	0.591
Posterior stroke (%)	1 (11)	1(6)	1.000

Effect of baseline cerebral microbleeds on long-term cognitive outcome

Baseline CMBs were associated with the prevalence of executive impairment at both baseline and follow-up, but not with impairment in other cognitive domains (Table 23). At follow-up, there were 7/9 [78%] patients with baseline CMBs impaired in executive functions vs 5/17 [29%] of patients without CMBs (p=0.038). Patients with CMBs had more domains impaired at follow-up compared to baseline, as did patients without CMBs (with CMBs: median 3 [0-5] at follow-up vs median 2 [0-2] at baseline, p=0.016; without CMBs: median 1.0 [0-5] at follow-up vs median 0 [0-5] at baseline, p=0.035). Comparisons between CMB and CMB-free groups at baseline and follow-up did not show differences in the median number of impaired domains (Table 23).

The presence of baseline CMBs was a predictor of executive dysfunction at follow-up, as were hypertension and premorbid intellectual functioning (Table 24). Baseline executive function was not a predictor of impairment in this domain at follow-up.

Table 24. Imaging and cognitive results for the original baseline cohort and the 5.7 year follow-up cohort

	Baseline (full cohort) [5]			Follow-up cohort		
	With CMBs (N=25)	Without CMBs (N=30)	p-value	With CMBs (N=9)	Without CMBs (N=17)	p-value
Imaging analyses						
Presence of cortical infarct (%)	6 (24)	5 (17)	NS	3 (33)	3 (18)	NS
Median total score of WML (range)	3 (0-16)	3 (0-22)	NS	9 (4-19)	6 (0-14)	NS
Cognitive impairment						
Current intellectual functioning (% impaired)	6 (24)	4 (13)	NS	2 (22)	5 (29)	NS
Verbal memory (% impaired)	2 (9)	3 (10)	NS	2 (22)	2 (12)	NS
Visual memory (% impaired)	1 (4)	5 (17)	NS	1 (11)	3 (18)	NS
Naming skills (% impaired)	3 (12)	6 (20)	NS	2 (22)	1 (6)	NS
Perceptual functions (% impaired)	3 (12)	1 (3)	NS	1 (11)	2 (12)	NS
Frontal executive functions (% impaired)	15 (60)	9 (30)	0.030	7 (78)	5 (29)	0.038
Speed and attention functions (% impaired)	10 (42)	13 (45)	NS	8 (88)	10 (59)	NS
Median N of cognitive domains impaired	2 (0-5)	0 (0-5)	NS	3 (0-5)	1 (0-5)	NS

Table 25. Regression analyses (unadjusted binary logistic regression) testing the effect of baseline variables including CMBs on executive impairment at the 5.7-year follow-up

	OR	95%CI	p
Age	1.10	0.99-1.22	0.056
Presence of CMBs	8.40	1.27-55.39	0.027*
Any new CMBs at follow-up	6.67	0.59-74.51	0.123
Hypertension	6.67	1.05-42.43	0.045*
Total score of WML	1.11	0.93-1.33	0.247
Years of education	0.91	0.77-1.08	0.277
Frontal-executive impairment at baseline	6.50	0.61-68.96	0.120
Premorbid intellectual functioning (NART)	0.90	0.82-0.99	0.034*

OR = Odds ratio; CI = confidence interval; NART = National Adult Reading Test.
Asterisks indicate statistically significant p values.

Changes in MRI findings over time

Of the 26 patients, 21 (8 with CMBs, 13 without) had follow-up MRI. Fifty percent of patients with CMBs [4/8] versus 8% [1/13] of patients without baseline CMBs developed new CMBs (p=0.047). The severity of WML increased in both groups; the difference was statistically significant only in patients without baseline CMBs (p=0.027). There were no new cortical infarcts.

7.2.5 Discussion

Our main findings were: (1) the finding of CMBs on first attendance at a stroke clinic predicted executive impairment at follow-up 5.7 years later; (2) patients with or without CMBs had more cognitive domains impaired at follow-up; (3) we observed worsening of MRI markers of small vessel disease (WML and new CMBs), but no new cortical infarcts.

CMBs have been found to be more common in patients with cognitive disorders including in a memory clinic cohort (Cordonnier et al., 2006), Alzheimer disease (Pettersen et al., 2008) and vascular dementia (Seo et al., 2007) than in healthy populations, but evidence of an independent effect of CMBs on the risk of cognitive impairment is limited. A case-control study of the full baseline data of the present cohort suggested that CMBs are selectively associated with executive impairment (Werring et al., 2004). The follow-up findings of the present study are consistent with these previous findings, although the

mechanisms remain speculative. The effect of CMBs on cognitive progression has been evaluated in CADASIL (Liem et al., 2009) and in MCI (Ayaz et al., 2010). To the best of our knowledge, no previous studies have investigated the prognostic value of CMBs and long-term cognitive outcome in stroke patients.

Both the CMB and CMB-free patient groups developed more impaired domains over the follow-up period. In parallel, we found MRI evidence of progressive small vessel damage: WML scores increased in both groups, whilst in those with baseline CMBs, 50% developed new CMBs. These observations suggest that cognitive deterioration after ischaemic stroke can result solely from progressive small vessel pathology rather than new cortical territorial infarcts, which did not occur in our cohort.

The strengths of our study are the use of a standardised neuropsychological battery by blinded neuropsychologists, of standardised MRI protocols and validated CMB and WML scales. The main weakness is the small sample size, meaning that our estimates of risk have wide CI, and our results must be considered to be preliminary rather than definitive. We did not have sufficient statistical power to investigate change in executive function over time or adjust our analysis of risk of follow-up cognitive impairment; nor could we investigate any association between imaging changes and cognitive progression. Furthermore, we were unable to characterise the subtype of cognitive disorder as no concomitant analysis using Pittsburgh Compound B-Positron Emission Tomography (PIB-PET) or single-photon emission computed tomography (SPECT) was presented.

7.2.6 Conclusions

In conclusion, our data support the hypothesis that CMBs are consistently associated with executive dysfunction, and may have prognostic relevance for long-term cognitive outcome, in stroke clinic patients. Further studies of the prognostic significance of CMBs for long term cognitive outcome in larger cohorts of stroke patients are warranted.

Chapter 8 - Cerebral Microbleeds and Acute Silent Ischaemia: Detection of acute silent ischaemia in a multi-centre cross-sectional MRI study of patients with intracerebral haemorrhage

8.1 Summary

Subclinical acute ischaemic lesions on brain MRI have recently been described in spontaneous ICH, and may be important to understand pathophysiology and guide treatment. The underlying mechanisms are uncertain. We tested the hypothesis that ischaemic lesions are related to MRI markers of the severity and type of small vessel disease (hypertensive arteriopathy or CAA) in a multicentre, cross-sectional study. We studied consecutive patients with ICH from 4 specialist stroke centres, and age-matched stroke service referrals without ICH. Acute ischaemic lesions were assessed on MRI (<3 months after ICH) using diffusion-weighted imaging. WML and CMBs were rated using validated scales. We investigated associations between DWI lesions, clinical and radiological characteristics. We included 114 patients with ICH (39 with clinically-probable CAA); and 47 age-matched controls. The prevalence of DWI lesions was 9/39 (23%) in probable CAA-related ICH versus 6/75 (8%) in other types of ICH (p=0.024); no DWI lesions were found in controls. DWI lesions were mainly cortical and were associated with mean WML score (OR 1.14 per unit increase, 95% CI 1.02-1.28, p=0.024) and the presence of strictly lobar CMBs (OR 3.85, 95% CI 1.15-12.93, p=0.029). Acute, subclinical ischaemic brain lesions are frequent but previously underestimated after ICH, and are three times more common in CAA-related ICH than in other ICH types. Ischaemic brain lesions are associated with WML and CMBs, suggesting that they result from an occlusive small vessel arteriopathy. DWI lesions contribute to the overall burden of vascular-related brain damage in ICH, and may be a useful surrogate marker of ongoing ischaemic injury from small vessel damage.

8.2 Rationale

ICH is the most disabling form of stroke, for which acute treatments are limited. There is an urgent need to better understand the pathophysiology of ICH to help enhance the development of new treatments. Most ICH (>75%) are classified as primary (spontaneous), due to rupture of small arteries of the brain, affected by two main disease processes: hypertensive arteriopathy and CAA. Hypertensive arteriopathy, characterised by lipohyalinosis and fibrinoid necrosis of small deep perforating arteries, is an important cause of ICH, particularly in deep brain structures, including the basal ganglia, brainstem and thalamus. CAA is defined by Aβ deposition in the media and adventitia of cortical and leptomeningeal small- to medium-sized arteries, arterioles and capillaries (Attems, 2005; Vinters, 1987) and is considered an important cause of lobar ICH, especially in the elderly.

DWI detects acute brain ischaemia with sensitivity approaching 100% (Davis et al., 2006). Recent studies using DWI have detected clinically unsuspected areas of acute ischaemia in ICH (Kidwell and Greenberg, 2009; Menon and Kidwell, 2009; Prabhakaran et al., 2010), but the mechanisms underlying these lesions are not clear. Ischaemia could, for instance, be due to an active occlusive small vessel arteriopathy (e.g. CAA); or due to cerebral hypoperfusion due to reduced BP in the context of a failure of autoregulation. It is of practical importance to establish the underlying mechanism because one of the few promising treatments currently under investigation for ICH is aggressive BP lowering to reduce haematoma expansion (Anderson et al., 2008; Delcourt et al., 2010). We reasoned that if ischaemic DWI lesions in ICH are due to occlusive small vessel damage, then they should be related to the severity of the underlying small vessel disease. We tested this hypothesis using MRI to detect acute ischaemic brain lesions and relating them to two MRI markers of cerebral small vessel disease: WML and cerebral CMBs. Since neuropathological analyses have identified asymptomatic ischaemic infarction as a common finding in the brain of patients with advanced CAA (Cadavid et al., 2000; Haglund et al., 2006b; Okazaki et al., 1979; Olichney et al., 1995; Soontornniyomkij et al., 2010), we also hypothesised that ischaemic lesions would be most common in ICH attributed to probable CAA, and in association with strictly lobar CMBs, a radiological marker for CAA.

8.3 Methods

8.3.1 Participants

We included patients within 3 months of spontaneous ICH consecutively referred to 4 specialist stroke centres in the United Kingdom and Belgium. The hospitals were: The NHNN, Queen Square (London), Addenbrookes' Hospital (Cambridge); Cliniques Universitaires Saint Luc (Brussels) and Cliniques Universitaires UCL de Mont Godinne (Yvoir). All centres have a policy of routine MRI for investigating the cause of spontaneous ICH. We identified all cases with spontaneous lobar ICH and appropriate MRI data available. Probable CAA was defined according to the Boston criteria: patients with two or more spontaneous strictly lobar ICH over the age of 55 years, without any other identified cause (Knudsen et al., 2001). The remaining patients with spontaneous ICH not fulfilling the criteria for CAA (e.g. one strictly lobar haemorrhage, mixed [deep and lobar] haemorrhages, and strictly deep haemorrhage[s]) were classified as non-CAA related. All cases were ascertained using overlapping methods from prospective clinical databases and radiological reports. Clinical information (demographics, vascular risk factors, use of antithrombotics) was obtained from prospective databases and medical records. A total of 422 patients with ICH were screened, of whom 129 were excluded because of diagnosis of non-spontaneous ICH (e.g. secondary to haemorrhagic infarction, aneurysms, tumours, cavernomas, arterio-venous malformations, coagulation disorders or use of oral anticoagulants, venous thrombosis or head injuries), and 179 because MRI with the necessary sequences of adequate quality was not available within 3 months of the acute ICH.

Age-matched controls were ascertained from a database of consecutive patients with suspected stroke attending the neurovascular clinic at NHNN but with a final non-stroke/TIA diagnosis for their presenting symptoms after full investigation. They were matched for age with the CAA-related ICH patients using an unbiased group matching process blinded to their final diagnoses and imaging findings.

8.3.2 Standard protocol approvals

The study received ethical approval by the NHNN and Institute of Neurology Joint Research Ethics Committee (approval number 07/Q0512/39; date of approval 22 October 2007), the Commission d'Ethique Biomédicale Hospitalo-facultaire of the Faculté de Médecine

(Cliniques Universitaires St Luc)(approval number 2008/ISJUIN/I 76; date of approval 23 June 2008), and of the Comité d'Ethique médicale of the Cliniques Universitaires UCL de Mont Godinne (approval number 53/2008; date of approval 08 July 2008).

8.3.3 MRI protocols

The MRI stroke protocol was standardised in each hospital. Imaging was at 1.5T field strength for the majority of patients (105/114, 92%) and included T1-weighted, T2-weighted, FLAIR, DWI and GRE T2* sequences.

National Hospital for Neurology and Neurosurgery, Queen Square

MRIs were carried at 1.5T field strength. Axial GRE T2* images were obtained using a General Electric Genesis Signa Scanner (GE Medical Systems, Milwaukee, WI) [TR 300 ms, TE 40 ms, FA 20°, field of view (FoV) 24 x 18, matrix 256 x 160, slice thickness 5 mm, gap 1.5 mm, number of excitations (NEX) 1] for 38 patients. Four patients were scanned on a Siemens Avanto Scanner (Siemens, Erlangen, Germany) [TR 800 ms, TE 26 ms, FA 20°, FoV 24 x 18, matrix 512 x 448, slice thickness 5 mm, slice gap 1.5 mm, NEX 1]. Axial DWI sequences were acquired using the following parameters: TR 1000 ms, TE 98.5 ms, *b* = 0/1000 s/mm^2 , slice thickness 5 mm, gap 0.5 mm, FoV 250 mm, matrix 256 x 256, NEX 1 (GE); TR 3200 ms, TE 78 ms, slice thickness 5 mm, gap 1.5 mm; FoV 250 mm, matrix 256 x 256, NEX 1 (Siemens).

Addenbrookes' Hospital, Cambridge

Twelve patients were scanned on a 1.5T GE Medical Systems Signa Excite scanner. GRE T2* sequences were obtained in the axial plane using the following parameters: TR 460-660 ms, TE 15 ms, FoV 22 cm, matrix 256x192-224, slice thickness 6 mm, gap 7mm, NEX 2. Four patients were scanned on a 3T GE Medical Systems Signa HDX scanner, with GRE parameters as follows: TR 600 ms, TE 15 ms, FoV 22cm, matrix 256x192, slice thickness 6 mm, gap 7 mm, NEX 1. Axial DWI sequences were acquired with the following parameters: at 1.5T, TR 10 000 ms, TE 61.6 ms, *b* = 0/1000 s/mm^2, slice thickness 5 mm, no gap, FoV 280 mm; at 3T, TR 6000 ms, TE 61.6 ms.

Cliniques universitaires Saint Luc, Brussels

At St Luc, axial GRE T2* sequences were acquired, with parameters as follows: 3T, TR 230 ms, TE 16 ms, slice thickness 4 mm, gap 0.4 mm; 1.5T, TR 230-240 ms, TE 50-70 ms, slice

thickness 5 mm, gap 1 mm. Axial DWI was acquired using the following parameters: TR 4500 ms, TE 94 ms, b = 0/1000 s/mm^2, slice thickness 5 mm, gap 0.5 mm; FoV 240 mm^2, matrix 256 x 256; TR 3312 ms, TE 93 ms, b = 0/1000 s/mm^2 , slice thickness 5 mm, gap 1 mm, in-plane resolution 1.88 x 2.33 mm^2 reconstructed to 0.94 mm^2; and TR 2907 ms, TE 55 ms, FA 90°, b = 0/1000 s/mm^2 , slice thickness 4 mm, gap 0.4 mm, in-plane resolution 1.8 x 2.27 mm^2 reconstructed to 0.9 mm^2.

Cliniques universitaires UCL de Mont Godinne

All MRIs were carried at 1.5T field strength. GRE T2* sequences were obtained in the axial plane on a Siemens Magnetom Symphony System [TR 921 ms, TE 22 ms, FoV 230 mm, matrix 256 (1.2 x 0.9 x 0.4 mm), slice thickness 4mm, slice gap 10%]; and an EPI sequence: TR 4750 ms, TE 46 ms, FoV 250, matrix 128 (2 x 2 x 3 mm), slice thickness 3mm, slice gap 10%. Axial DWI sequences were acquired with the following parameters: TR 4900 ms, TE 93 ms, *b* = 0/1000 s/mm^2 , slice thickness 4 mm, gap 0.4 mm, FoV 250 mm, matrix 256 x 256.

8.3.4 Imaging analysis

All images were analysed by a clinical neurologist (SMG), blinded to clinical information. Acute ischaemic lesions were identified as bright areas on DWI sequences and corresponding dark areas on ADC maps by SMG and reviewed by a senior vascular neuroradiologist (HRJ) to reach consensus, blinded to other imaging sequences and clinical details. DWI lesions in close proximity (<20mm) to an ICH were excluded. The locations of DWI lesions were classified as cortical or cortical-subcortical (including cerebellum) or deep (including brainstem). We recorded the presence and measured the number of acute symptomatic ICHs and the median total number of ICH on MRI. ICH distribution was classified as lobar (including cerebellum) and deep (including brainstem). SMG rated the presence, number and distribution of CMBs on GRE T2* images using the CMB anatomical rating scale (MARS) (Gregoire et al., 2009). WML were rated on a 4-point scale, with scores ranging from 0 to 3 (Wahlund et al., 2001) on sagittal T1, coronal FLAIR and axial T2-weighted MR images. The presence of subarachnoid blood, intraventricular haemorrhage and superficial siderosis were also recorded.

8.3.5 Statistical analysis

We compared the characteristics of patients with probable CAA-related ICH and the remaining patients with spontaneous ICH. Population characteristics were compared using

the Chi-square test and Fisher's exact test for categorical variables. Continuous variables were analysed using independent samples *t*-test (when the data distribution was normal) and the Mann-Whitney U tests (when the distribution was non-normal). We used univariate binary logistic regression to test for the factors associated with DWI lesions. The variables entered into the multivariate regression analysis model were variables thought to be potential confounders (age, sex) and had been significant in univariate regression. The threshold of statistical significance was set at $p<0.05$ for all analyses.

8.4 Results

The final cohort consisted of 114 patients with ICH, 39 of whom fulfilled the criteria for probable CAA; and 47 age-matched controls. The clinical and radiological characteristics of the ICH cohort are reported in Table 25. The ICH patients excluded from the study were not significantly different from those included in measures of ICH severity including: median ICH volume 17.7 cm^3 [range 0.40-73.56 cm^3] in patients excluded versus 10.5 cm^3 [range 0.31-50.00 cm^3] in patients included, p = 0.93; median GCS on admission: 14 [range 9-15] in patients excluded versus 15 [range 6-15] in patients included, p = 0.691. Patients excluded had a higher prevalence of symptomatic lobar ICH (excluded: 82% lobar versus included 54% lobar, p = 0.002).

In our age-matched control group, the following prevalences of vascular risk factors were found: hypertension 83%, diabetes 13%, previous history of stroke 29%, use of antithrombotics 46%. Controls had the following final diagnoses: syncope (N=7 [17%]), vestibular disturbance (N=7 [15%]), asymptomatic carotid stenosis (N=6 [13%]), transient global amnesia (N=4 [9%]), functional (N=4 [9%]), small vessel disease without acute stroke (N=4 [9%]), migraine (N=4 [9%]), memory problems (N=2 [4%]), incidental cerebral infarction (N=1 [2%]), other (N=7 [15%]).

Table 26. Characteristics of patients with probable CAA compared with patients with other causes of spontaneous ICH

	Probable CAA (N=39)	All other ICH‡ (N=75)	p
Clinical demographics			
Median age in years (range)	74 (49-90)	69 (49-93)	0.094
Sex, female (%)	14 (36)	30 (40)	0.669
Hypertension (%)	24 (62)	64 (85)	0.013*
Smoking history (%)	11 (28)	23 (31)	0.884
Diabetes (%)	6 (15)	13 (17)	0.859
Statins on admission (%)	8 (21)	16 (21)	1.000
Previous history of ischaemic stroke or TIA (%)	5 (13)	7 (9)	0.549
On antithrombotics (%)	11(28)	26 (35)	0.537
First ever ICH (%)	20 (51)	61 (81)	<0.001**
Median interval between ICH and MRI in days (range)	8 (0-76)	5 (0-84)	0.300
Radiological characteristics			
Median total score of WML (range)	9 (2-26)	9 (0-20)	0.505
Presence of cortical infarcts (%)	2 (9)	4 (10)	1.000
Presence of lacunar infarcts (%)	9 (41)	21 (55)	0.284
Presence of CMBs (%)	35 (90)	42 (56)	<0.001**
Presence of multiple ICH (%)	19 (49)	14 (19)	<0.001**
Median total number of ICH on MRI (range)	1 (0-5)	1 (1-3)	0.002**
Presence of SAH or IVH (%)	15 (38)	11 (15)	0.004**

BP = blood pressure; TIA = transient ischaemic attack; WML = white matter lesions; SAH = subarachnoid haemorrhage; IVH = intraventricular haemorrhage
*p< 0.05; **p< 0.01
‡ Possible CAA and non-CAA

Examples of GRE T2* MRI studies of two non-CAA patients are shown in Figure 52: one with a mixed distribution of haemorrhages (a large deep symptomatic haemorrhage with both deep and lobar cerebral CMBs); and one with exclusively deep haemorrhages. There were 33 patients with multiple (≥2) ICHs (29%). In these, all ICHs identified on MRI (apart from the symptomatic lesion) occurred at previous remote times, except in 2 patients with simultaneous multiple ICHs at presentation.

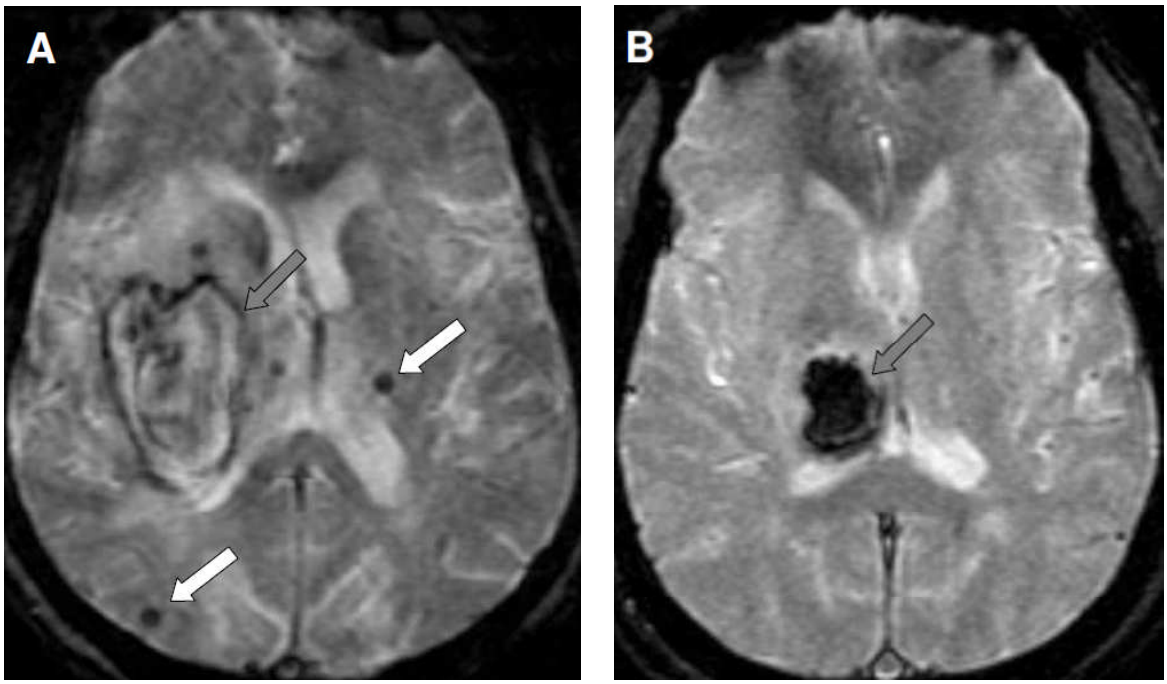


Figure 52. GRE T2* images of non-CAA cases.

A. Bleeds with mixed distribution. The GRE T2* image shows a deep ICH in the right basal ganglia (grey arrow), and CMBs in the left internal capsule and right occipital lobe (white arrows). **B.** Deep bleeds only. The GRE T2* image shows an ICH in the right thalamus (grey arrow).

8.4.1 Prevalence of DWI lesions

The prevalence of DWI lesions was as follows: ICH patients overall = 15/114 (13%); probable CAA = 9/39 (23%); all other ICH = 6/75 (8%); controls = 0/47(0%). Two patients had two DWI lesions; the rest had a single DWI lesion. Representative examples of DWI lesions are shown in Figure 53. DWI lesions were more common in patients with probable CAA compared with patients with other causes of ICH (p = 0.024). DWI lesions were most commonly found in lobar cortical-subcortical areas (N = 12, 75%), especially the frontal lobes (N = 6, 38%). DWI lesions were all round or ovoid; their size was 0.42-1.46 cm diameter in patients with probable CAA, and 0.25-1.50 cm in patients with other causes of spontaneous ICH (p = 0.328). Half of the MRI scans were done within 1 week of the presenting ICH (63/114 [55%]); and 99/114 [87%] within 1 month. The longest interval between presenting ICH and MRI was 84 days. About one-third of DWI lesions were detected within 1 week (6/17 [35%]); 14/17 (82%) were detected within 1 month. The latest DWI lesion detected was at 64 days after the presenting ICH. No DWI lesions were associated with any new clinical symptoms.

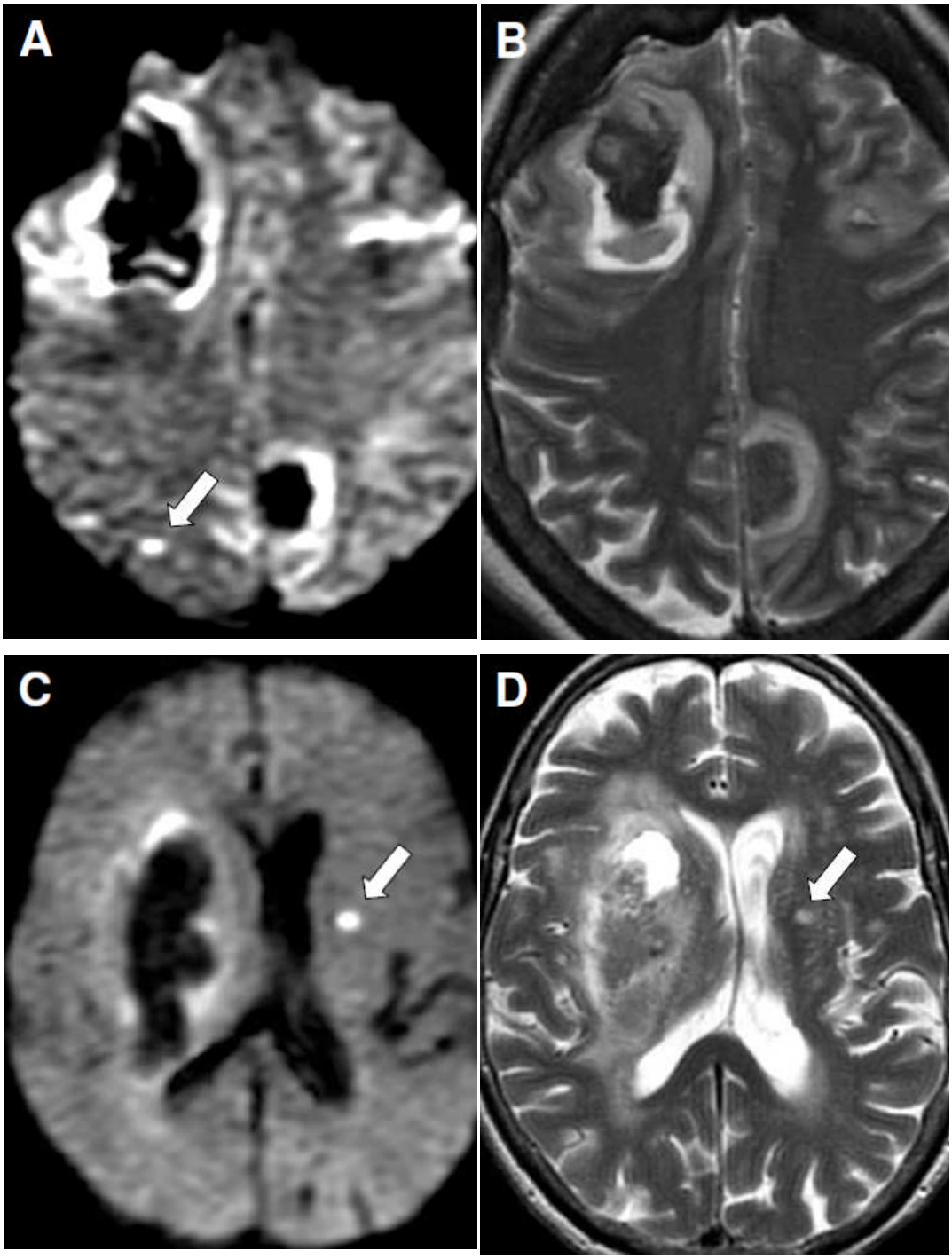


Figure 53. Examples of positive DWI lesions

Top panels **(A)** and **(B)** show probable CAA related ICH: **(A)** DWI sequence showing presence of small ischaemic lesion in the right parietal lobe (white arrow) in the presence of 2 lobar ICH, frontal and parietal, suggestive of CAA; **(B)** corresponding T2-weighted sequence. Bottom panels **(C)** and **(D)** show non-CAA related ICH: **(C)** DWI sequence showing presence of small lacunar ischaemic lesion in the left lentiform nucleus (white arrow) in presence of deep ICH; **(D)** a small infarct is visible on the corresponding T2- weighted sequence (white arrow).

Patients with DWI lesions had more severe WML (mean total score 12 [range 3-17] versus 8 [range 0-26], p = 0.007), and a higher prevalence of lobar CMBs (14/15 [93%] versus 57/99 [58%], p = 0.008), but not deep CMBs (4/15 [27%] versus 28/99 [28%]) (see Table 26). There was no difference in admission systolic or diastolic BP between patients with and without DWI lesions.

Table 27. Characteristics of patients with and without DWI lesions

	DWI (+) lesion (N=15)	DWI (-) lesion (N=99)	p
Clinical demographics			
Median age in years (range)	73 (49-88)	71 (19-93)	0.973
Sex, female (%)	4 (27)	10 (10)	0.308
Hypertension (%)	10 (67)	78 (80)	0.307
Median interval between ICH and MRI in days (range)	10 (0-64)	4 (0-84)	0.471
Mean SBP in mmHg (SD)	162 (37)	161 (29)	0.840
Mean DBP in mmHg (SD)	85 (21)	87 (16)	0.693
Smoking history (%)	8 (53)	26 (27)	0.067
Diabetes (%)	4 (27)	15 (16)	0.284
Statins on admission (%)	4 (27)	20 (21)	0.736
Previous history of ischemia or TIA (%)	3 (20)	9 (9)	0.202
On antithrombotics (%)	3 (20)	34 (36)	0.229
Diagnosis of probable CAA (%)	9 (60)	30 (30)	0.024*
Radiological characteristics			
Median total score of WML (range)	12 (3-17)	8 (0-26)	0.007**
Presence of cortical infarcts (%)	0 (0)	6 (11)	1.000
Presence of lacunar infarcts (%)	4 (80)	26 (47)	0.353
Presence of multiple ICHs (%)	5 (33)	28 (29)	0.764
Median total number of ICH on MRI (range)	1 (1-3)	1 (0-5)	0.581
Presence of SAH or IVH (%)	5 (33)	21 (21)	0.327
Presence of strictly lobar CMBs including cerebellum (%)	14 (93)	57 (58)	0.008**
Presence of deep CMBs including brainstem (%)	4 (27)	28 (28)	1.000
Median total number of strictly lobar CMBs (range)	4 (0-14)	1 (0-294)	0.028*
Median total number of deep CMBs (range)	0 (0-14)	1 (0-14)	0.983

BP = blood pressure; SD = standard deviation; TIA = transient ischaemic attack;
WML = white matter lesions; SAH = subarachnoid haemorrhage;
*p< 0.05; **p< 0.01

8.4.2 Estimated incidence of DWI lesions

Assuming that DWI lesions remain detectable for about ~10 days after stroke (Burdette et al., 1998), then the estimated annual incidence can be calculated as:

$$\textit{Estimated annual incidence} = \frac{no\ of\ DWI\ (+)\ lesions}{no\ of\ patient\ population\ found} \times \frac{365\ (days\ per\ year)}{duration\ of\ DWI\ lesions\ (days)}$$

For a consistent incidence of DWI lesions per year, the estimated annual incidence in the whole cohort of ICH patients would be (16/114) x (365/10) = 5.1 silent infarctions per person-year; in probable CAA-related ICH the estimated annual incidence = (9/39) x (365/10) = 8.4 silent infarctions per person-year.

8.4.3 Factors associated with DWI lesions

In univariate regression analysis, significant predictors of DWI lesions were smoking history, diagnosis of probable CAA, total score of WML, and the presence of strictly lobar CMBs (Table 27).

Table 28. Univariate regression analysis testing the factors associated with DWI lesions

	OR	95% CI	p
Clinical characteristics			
Age in years	1.00	0.96-1.05	0.941
Sex, male	0.54	0.16-1.80	0.314
Hypertension	0.49	0.15-1.59	0.234
Number of BP medications at time of ICH	0.88	0.51-1.53	0.648
Smoking history	3.08	1.01-9.33	0.047*
Diabetes	1.96	0.55-6.99	0.298
High cholesterol	0.77	0.39-1.49	0.438
On antithrombotics	0.45	0.12-1.70	0.238
Classification of ICH mechanism			
Diagnosis of probable CAA	3.45	1.13-10.56	0.030*
Radiological characteristics			
Total score of WML	1.13	1.02-1.26	0.019*
Presence of multiple ICH	1.23	0.39-3.93	0.724
Presence of lacunar infarcts	4.46	0.47-42.5	0.194
Presence of CMBs			
Presence of CMBs	8.00	1.01-63.38	0.049*
Presence of strictly lobar CMBs, including cerebellum	10.32	1.31-81.55	0.027*
Presence of deep CMBs, including brainstem	0.92	0.27-3.14	0.897

*p< 0.05; **p< 0.01

In multivariate analysis, DWI lesions were associated with both mean WML score (OR 1.14 per unit increase, 95% confidence interval [CI] 1.02-1.28, p = 0.024) and the presence of strictly lobar CMBs (OR 3.85, 95% CI 1.15-12.93, p = 0.029) (Table 28). These associations remained significant after adjustment for age and gender. Adjusting the analysis for variation in magnetic field strength (1.5 or 3T) did not substantially weaken the association between lobar CMBs and the presence of DWI lesions (adjusted OR 3.73, 1.11-12.57, p = 0.034).

There was no statistically significant difference in the median ICH volume between DWI-positive and DWI-negative groups: for DWI-negative patients, median ICH volume was 10.25cm^3 [range 0.31-37.63], whilst in DWI-positive patients, median ICH volume was 12.09cm^3 [range 0.58-50.00] (p=0.892).

Table 29. Multivariate regression analysis showing the factors associated with DWI lesions*

	O.R.	95% CI	p
Characteristics			
Mean white matter change score	1.14	1.02-1.28	0.024
Strictly lobar CMBs	3.85	1.15-12.93	0.029
Age (years)	0.97	0.92-1.03	0.374
Sex	0.62	0.17-2.29	0.469

* Hosmer-Lemeshow goodness of fit: Chi-Square 4.986, sig. 0.759
Positive predictive value: 100%
Negative predictive value: 88%

8.5 Discussion

In this study, we frequently detected subclinical acute ischaemic brain lesions in ICH patients; they were about three times as common in probable CAA-related ICH compared with the other cases of ICH. We found no DWI-hyperintense lesions in our age-matched controls despite them having a similar or higher prevalence of conventional vascular risk factors to the ICH patients. DWI lesions were associated with markers of small vessel damage including total WML and the presence of lobar CMB, but not with admission BP.

Overall, DWI-positive lesions were predominately located in lobar areas, mainly in the cortical-subcortical areas of the frontal and temporal lobes. They were small and mostly ovoid or round in shape. The distribution and morphology of the detected DWI lesions correlates well with the 'icroinfarcts' identified in neuropathological studies of patients with CAA (Cadavid et al., 2000; Haglund et al., 2006b; Okazaki et al., 1979). Since these lesions were associated with markers of small vessel damage severity (WML and CMBs), rather than conventional risk factors, they probably represent acute small vessel infarcts related to small vessel arteriopathy, especially CAA. About a third of silent ischaemic lesions were detected within one week after ICH, and ~80% of them within one month, which suggests a possible early peak followed by a descending incidence over time, but we were unable to investigate this further as we did not acquire repeat imaging studies in this cohort.

To our knowledge, only one imaging study has investigated the presence of silent ischaemic lesions in CAA (Kimberly et al., 2009); DWI-hyperintense lesions were identified in 12 out 78 subjects (15%) with probable or definite CAA, while no lesions were found in a control group of 55 subjects with Alzheimer disease or MCI (p=0.001). In agreement with our findings, the authors did not find an association between DWI lesions and conventional risk factors. In contrast to our results, they found no association between DWI lesions and WML, but this study did not include any ICH cases not attributed to CAA so was not representative of the full spectrum of spontaneous ICH. Another recent study found DWI lesions in 23% of patients in a cohort of 118 patients with ICH scanned within one month, and found that DWI lesions were associated with aggressive BP lowering (Prabhakaran et al., 2010), but this study did not investigate the relationship of DWI lesions to MRI markers of small vessel disease and did not use formal criteria to define CAA.

What are the implications of our findings on the understanding of ICH pathophysiology? We demonstrated a high prevalence of silent acute ischaemic events within the first 3 months after CAA-related ICH at a mean time of 8 days. More than 80% of the lesions occurred within 1 month post-ICH. Since DWI lesions are positive only for about ~10 days (Burdette et al., 1998) and were still detectable in our study up to about ~2 months (Table 26), these lesions are likely to be a dynamic persisting phenomenon in ICH patients, and a common feature of the underlying arteriopathy. Ischaemia may therefore be a previously unrecognised cause of progressive disability with possible relevance for clinical outcome after ICH. The lack of association with admission BP suggests that hypoperfusion resulting from low systemic BP is unlikely to have caused these ischaemic lesions, although we do not have detailed information on BP control. Imaging data suggest that autoregulation is largely preserved in acute ICH, supporting this conclusion (Reinhard et al., 2010). Indeed, lowering BP is one of the most promising available treatments in acute ICH, having been shown to reduce haematoma expansion in a randomised trial (Anderson et al., 2008); a further large study is underway (INTERACT 2) (Delcourt et al., 2010). Antihypertensive treatment is also an important chronic treatment for patients following ICH (Arima et al., 2010; Progress Collaborative Group, 2001), but the effects on vascular damage from silent ischaemic lesions is unknown.

Our finding of a significant association between DWI lesions and lobar CMBs, and WML severity adds to the evidence that, although generally considered to be a haemorrhagic disorder, CAA is also characterised by frequent small infarcts (Menon and Kidwell, 2009; Soontornniyomkij et al., 2010). Since WML severity increases with increasing CMB burden in CAA cohorts and thus with the severity CAA-related microvasculopathy (Greenberg et al., 2004a), infarcts seem to relate to the severity of the underlying small vessel damage. Furthermore, the relation between DWI lesions and presence of CMBs suggests shared pathophysiological pathways for haemorrhagic process and ischaemia. In CAA, amyloid-β is deposited in small arteries and arterioles, causing thickening of the vessel wall and lumen restriction (Olichney et al., 1995), and endothelial/vascular smooth muscle dysfunction (Dotti and De Strooper, 2009). These changes can not only cause vessels to become brittle and prone to microaneurysm formation and blood leakage (Attems et al., 2011), but also to impaired local regulation of cerebral blood flow and thus small-vessel or capillary occlusion (Smith and Greenberg, 2009). The use of PET with amyloid-binding ligands (such as Pittsburgh B compound) will help to determine whether a regional correlation exists between amyloid deposition and ischaemia, as in CAA-related haemorrhage (Dierksen et

al., 2010). Other mechanisms such as blood-brain barrier disruption and active inflammation could also contribute to this active vasculopathic process. Taken together with recent reports of the development of CMBs after IS, our data suggest a previously unsuspected and intriguing interplay between the haemorrhagic and ischaemic components in small vessel diseases (Kidwell and Greenberg, 2009), suggesting a dynamic continuum between 'microbleeding' and 'microinfarction'.

DWI lesions were found in only 8% of patients with ICH who did not fulfill the criteria for probable CAA. This group had a higher prevalence of hypertension and a mixed pattern of CMBs (lobar and deep) suggesting that microinfarction can also result from hypertensive arteriopathy. The association between asymptomatic acute infarcts and hypertensive small vessel disease was suggested in previous studies, although no definite correlation with hypertension was found (Jouvent et al., 2011; Kang et al., 2004; O'Sullivan et al., 2003). In our study, it is possible that some of the ischaemic lesions in the non-CAA ICH group may have in fact been due to the presence of CAA, hypertensive arteriopathy and CAA often co-exist. Moreover, although the Boston criteria have been shown to have excellent specificity for CAA, the sensitivity is low, with a negative predictive value of 39%, so that some lobar ICH patients not fulfilling the Boston criteria are likely to have a degree of CAA pathology (Knudsen et al., 2001); this could have weakened the association we found between probable CAA and DWI-lesions.

The association between DWI lesions and WML burden suggests that at least some WML could be due to accumulated microinfarcts (Smith, 2010), or chronic hypoperfusion. Another potential mechanism through which CAA-affected cortical and leptomeningeal vessels can damage white matter is by the entrapment of amyloid-β in perivascular spaces, impairing interstitial drainage pathways from white matter otherwise not directly affected form CAA pathology (Roher et al., 2003).

Our study has several strengths: the systematic evaluation of MRI scans by one trained rater using validated scales, the concomitant use of DWI and ADC maps to assess the presence of silent ischaemia and the review of all positive scans by an experienced neuroradiologist. It suffers from limitations due to the lack of pathological confirmation of the ischaemic nature of the DWI lesions and of the CAA pathology as a cause of ICH. In all hospital-based ICH MRI studies, it is not possible to completely rule out bias as the most severe cases will suffer early mortality or be unable to tolerate an MRI examination. Thus,

in the present study, there is likely to be ascertainment bias towards a less severely impaired cohort. However, we found no significant differences in measures of ICH severity (including hematoma volume and GCS scores on admission) between included and excluded subjects. Patients excluded had a higher prevalence of symptomatic lobar ICH, which could lead to an underestimation of the association between DWI lesions and CAA-related ICH. Finally, we could not rule out the confounding effect of a direct influence of the acute ICH in causing ischaemia as we did not include a group of patients with CAA without symptomatic lobar haemorrhage, but we excluded any DWI lesions in close proximity to the symptomatic ICH, and found no relationship between ICH volume and the presence of DWI lesions, making this unlikely.

Our findings raise clinical questions, especially the dilemma about the use or avoidance of antithrombotics in patients with ICH. Although the risk of anticoagulation likely overweighs the benefit in most patients with ICH due to CAA, the risk versus benefit of antiplatelet agents is less clear (Biffi et al., 2010b; Rosand et al., 2000). The presence of DWI lesions may identify a subgroup of ICH patients who might have greater benefit from antithrombotic treatment, but clinical studies are needed to address this question.

8.6 Conclusions

Our study clearly points to a previously unsuspected high prevalence of acute ischaemia in patients within 3 months of ICH, but longitudinal studies with serial clinical examinations and imaging are also needed to further characterise the dynamics, associations and clinical impact. Further studies will determine whether the detection of DWI positive lesions in CAA can be used as a surrogate marker with value in diagnosis or assessing the severity and progression of the disease.

Chapter 9 - Discussion and Conclusions

9.1 Discussion

CMBs were first described in the mid-1990s as small hypointense dots detected *in vivo* on T2*-weighted MR sequences (O'Sullivan et al., 2002; Schrag et al., 2010), and with a physical size of less than a millimetre they were thought to be clinically silent However, they have emerged as an important new imaging marker of cerebral small vessel diseases (Greenberg et al., 2009b; Offenbacher et al., 1996) and their impact is increasingly recognised; they may be associated with an increased risk of haemorrhagic stroke, IS and dementia (Charidimou et al., 2013a; Cordonnier and van der Flier, 2011; Werring et al., 2004). The sequences sensitive to CMBs are now used in routine clinical settings, and the sophisticated detection methods and ageing population mean that CMBs are encountered in an ever-increasing number of patients: from population-based samples of healthy elderly people, to stroke populations and patients with cognitive impairment. Therefore, there is an urgent need to establish their pathophysiology and clinical significance.

In the first three chapters I covered the historical context of CMBs and the mechanisms underlying CMBs from histopathological studies, thereby demonstrating their close association with two underlying pathological processes, hypertensive arteriopathy and CAA. I described the technical aspects of their detection, definition and mapping in the brain and showed that they are readily detected on iron-sensitive MRI sequences. I reviewed the epidemiology of CMBs and discussed their association with clinical and imaging factors in different populations, including patients with cognitive impairment or users of antithrombotic medications. In the later chapters I reported the findings of our main studies.

In this chapter I first summarise our main findings and discuss their relevance in the context of current knowledge, before raising the questions that are the most relevant in CMB research, and attempting to provide answers in the perspective of our findings and the available literature. Finally, I highlight points and areas where knowledge in CMBs is lacking and suggest directions for future research studies.

9.1.1 Summary of our main findings

Our studies resulted in some novel observations which are summarised below.

Development of an optimal rating method

CMBs have been studied increasingly often over the past decade, and when we started this work there was a need for a standardised assessment of CMBs in order to compare study results and pool data across studies and centres. To address this issue we developed a new CMB rating scale (see Chapter 4). We designed and tested the MARS on a population of 301 patients referred to our stroke service and performed reliability assessments for two raters with different levels of experience in neuroimaging and across two different MRI sequences (TE 26 ms and TE 40 ms). We found that MARS had good-to-very-good inter- and intra-rater reliability in most locations of definite CMBs in the brain. However, in regions where CMB mimics are most frequent (basal ganglia, cerebellum), reliability was slightly lower. We also compared our scale with the only other existing scale (BOMBS) on a subsample of 100 patients taken from the same cohort. Slightly different reliabilities were obtained for MARS and BOMBS, with a trend towards a better reliability of MARS (kappa 0.69 versus 0.68, with overlapping CI). The main focus of our scale was to detail the anatomical distribution of CMBs in order to assess their clinical significance in relation to their location in specific brain lobes and regions. The anatomical distribution of CMBs is crucial for studies investigating the relevance of CMBs in cerebrovascular diseases (CAA, hypertensive small vessel disease) and in cognitive dysfunction. Our ‘deep’ and ‘lobar’ boundaries were based on the potential to differentiate hypertensive small vessel disease and hypertensive haemorrhage (likely deep distribution) from CAA (likely lobar distribution). The other main focus of the scale was to provide a set of criteria by which to define CMBs because at the time their radiological characteristics for reporting had not been standardised. Furthermore, we provided a range of MRI criteria allowing CMBs to be categorised as ‘definite’ or ‘possible’, the latter being extremely frequent (up to 1/3 of all scored CMBs) and recommended for maximal reliability reporting definite CMBs only. An increasing number of studies show that quantification of CMBs may be of clinical relevance, in terms of the risk of recurrent ICH and subsequent cognitive impairment. We therefore assessed the reliability of our scale not only for CMB presence, but also for CMB number. However, we may have underestimated the number of CMBs on our scans due to the fact that we used radiological methods routinely used in most centres (GRE T2* at 1.5T in 2D, no post-processing techniques). Although we used standardized MRI parameters, the majority of

patients were scanned using low resolution sequences, without post-processing techniques. Also there was heterogeneity in the MRI parameters between centres. However, only a few patients were scanned on a higher resolution scanner at each centre, and we do not think that this would have influenced the results. Using higher resolution scanner may have introduced potential bias towards an overestimation of the reliability of MARS, as suggested by the slightly improved reliability using a sequence with a lower TE. New MRI methods with better resolution (including higher field strength and 3D images) will improve the depiction of CMBs, although whether these techniques will improve the reliability in CMB rating remains to be determined. Recent evidence suggests that SWI has greater reliability and sensitivity for detecting CMBs than GRE T2* MRI (Cheng et al., 2013). Methods based on automatic detection will help to increase the reliability of CMB ratings compared with manual ratings. Finally, our assessment of MARS was a limited validation of the scale only, due to the fact that only reliability was assessed, and that we had only 2 raters from the same department. We recommend the use of standardized imaging and clear definition of definite CMBs when rating CMBs in studies. These should involve two experienced raters and cases of disagreement should be reviewed by a senior neuroradiologist.

Antiplatelet-associated intracerebral haemorrhage risk

In Chapter 5 we demonstrated a strong independent association between CMBs and increase risk of ICH when on antiplatelet treatment, which has potential implications for the use of antiplatelet agents in patients with CMBs. The number of CMBs was significantly associated with ICH in patients on anti-platelet treatment, suggesting that the risk of ICH could outweigh the benefit of treatment with aspirin in patients with numerous CMBs. The novel aspect of our study was in showing that CMBs are more common in antiplatelet-related ICH compared to a matched antiplatelet user group without ICH, and in a second case group of patients with spontaneous ICH unrelated to antithrombotic agents. We were the first group to include these two comparison groups (a control group of aspirin users without ICH and a second case group of spontaneous ICH). Because CMBs are known to be associated with ICH and aspirin use is also associated with ICH, these two comparison groups allowed us to control for the effect of these confounding associations in the link between CMBs and ICH in patients on anti-platelet treatment. Another important contribution of our study is that it was the first to highlight CMBs as a potential risk factor for ICH in a non-Asian cohort. It is likely that patients with non antiplatelet-associated ICH

had a higher prevalence of CMBs because they had underlying CAA which might have contributed to the incidence of ICH without the use of antiplatelets. Although our study did not completely eliminate selection bias, mainly related to the fact that patients with more severe ICH did not have an MRI and might have been excluded, it is the closest that any ethical study can get to including a representative ICH population. Our study design of matching cases to controls for all potential confounding factors is a powerful way to investigate how CMBs relate to ICH in aspirin users. Critics may comment that we did not fully investigate the potential interaction between CMBs and aspirin use for ICH because there were no controls with neither ICH nor aspirin use. However, it would have been impossible to find non aspirin using controls that could be matched with our case group for age, hypertension and other vascular risk factors. Finally, we could not prove causality between antiplatelet therapy, and anti-platelet associated ICH. Due to these limitations and also to our small sample size, we acknowledge that our study does not make a strong enough case to recommend completely avoiding antiplatelet agents in patients with IS, or even in patients with CAA – who are at even greater risk of ICH. Given the limitations of methodology, our findings need to be investigated further before advocating a change in practice. However, our data did suggest that caution is advisable when prescribing antiplatelet agents in such patients, and that the finding of CMBs should be considered as part of the clinical decision making process.

Temporal changes of cerebral microbleeds

In Chapter 6 we presented the results of a five year clinical and imaging follow-up study in a small sample of patients with TIA and IS. We assessed the incidence of new CMBs and predictors of new CMBs in this specific population. Our results showed that half the patients with CMBs developed new lesions after five years, and that the main risk factors for developing new CMBs was the presence of CMBs at baseline and baseline systolic BP. All the patients who developed new CMBs had a baseline systolic BP of above 150 mmHg. Our findings suggest that CMBs are a dynamic process, with a burden that increases over time in relation to increased baseline BP. This observation had not been made in stroke cohorts prior to publication, nor had a link been made between a high baseline systolic BP and CMB accumulation. Our findings are consistent with other markers of small vessel disease progression, which strengthen the role of CMBs as another marker of small vessel disease. They emphasises the need to vigorously treat hypertension in patients with CMBs, as uncontrolled hypertension may contribute to the multiplication of CMBs and cause

potentially devastating effects, including ICH and further ischaemic events. Another striking finding was that most patients who developed new CMBs remained clinically stable over the follow-up period, suggesting that CMBs could be used as a marker to monitor the progression of small vessel pathology in the brain. Lacunar infarcts and WML were not associated with the development of new CMBs in this study. Our conclusions were drawn from a small cohort (only 8 patients had new CMBs) which limits the power of our study. Also, lobar CMBs were predominant in our cohort, and in a higher proportion than in other IS cohorts (Han et al., 2009), which might have biased our findings towards detecting more lobar CMBs at follow-up. However, our study was the first to show a relationship between hypertension and further CMB formation in patients with ischaemic cerebrovascular disease. If confirmed in larger cohorts then this observation has considerable implications for the treatment of stroke patients.

Contribution of cerebral microbleeds to cognitive impairment

The effects of CMBs on cognition have been studied in various population groups, from healthy elderly community subjects to subjects with memory impairment (Cordonnier et al., 2006; Poels et al., 2012a; Yakushiji et al., 2008). Their prognostic relevance for future cognitive impairment in stroke patients is uncertain due to the paucity of studies undertaken within this population group, and to the fact that the majority of the previous studies were cross-sectional and did not look at the effects of CMBs on cognitive functions over time. The first preliminary report by Werring et al. (2004) demonstrated an independent association of CMBs with executive impairment in a small (55 patients) cohort of stroke patients against matched controls. In Chapter 7.1 we confirmed this observation in a larger cohort of 320 patients. We demonstrated that this effect is largely driven by lobar CMBs, a marker for the presence of CAA. We found that patients with strictly lobar CMBs were more than twice as likely to have impaired executive functions. The presence of more than five CMBs in a strictly lobar distribution increased the risk of executive impairment by a factor 13, suggesting a role for CAA in VCI. At the time of submitting our paper, further reports were published supporting our preliminary hypothesis linking lobar CMBs and CAA with cognitive impairment, although the methods of assessing neuropsychological functions differed (Arvanitakis et al., 2011). In the absence of data from large prospective cohorts this was the best study model to test CMBs for cognitive impairment as we included all available data and used standardised imaging and comprehensive neuropsychological protocols. We believe that our population is similar to

other populations of stroke patients with ischaemic cerebrovascular disease. Patients with a non-stroke diagnosis were excluded for clarity and generalizability of the findings to other stroke cohorts. The study was undertaken in a stroke population with a high burden of vascular disease; in the absence of comparisons between representative matched population samples, this does not make our findings generalizable to non stroke populations. We do not know if stroke populations behave differently in terms of cognitive deficits than non stroke populations with similar CMB load. It is possible that using optimised imaging sequences (higher resolution with 3D, higher magnetic field strength [7T versus 1.5T], improved image processing techniques such SWI) we would have detected more CMBs but we would not expect these to alter associations with cognition.

In Chapter 7.2 we demonstrated that CMBs are consistently associated with executive impairment after six years in a small longitudinal study. Our findings demonstrated a trend towards a greater proportion of patients exhibiting a deterioration of their executive functions over time. None of the patients with CMBs recovered normal executive functions; they either remained impaired or developed new impairments. Likewise, there was an increase in the number of cognitive domains impaired at follow-up compared with baseline for both patients with and without CMBs, suggesting that cognitive impairment extends beyond executive functions over time and becomes more severe and widespread in the long term. This was the first longitudinal study testing detailed cognitive functions in stroke patients at baseline and follow-up. Several limitations weaken our paper, including the lack of follow-up imaging analyses to further specify the type of cognitive disorder, investigate the role of CMB distribution (lobar versus deep) on cognitive function, and study the causative link with CMBs. The small sample size (only 9 patients with CMBs had neuropsychological follow-up) precluded analyses controlling for all confounding factors, such as premorbid intellectual functioning, and resulted in wide CIs. Furthermore, we could not use continuous data, which would have given more power to our analyses and would have allowed an analysis of change of cognition. However, we believe that our findings are generalisable and that they will be replicated in other, larger stroke cohorts.

Association with acute ischaemia

In CAA, a disease strongly associated with ICH and the presence of lobar CMBs, several neuropathological studies have demonstrated that asymptomatic ischaemic infarction is a key pathological feature, especially in patients with advanced disease (Vinters, 1987). In Chapter 8 we investigated the presence of acute ischaemia on DWI MRI in patients with

acute ICH consistent with a diagnosis of CAA. We described the presence of lesions exhibiting diffusion restriction (acute ischaemic lesions) in 114 patients with acute ICH and assessed their relation to markers and severity of small vessel disease. We found DWI positive lesions to be most frequent in CAA-related ICH and to be related to the severity of WML and CMBs. We concluded that the presence of acute ischaemic lesions in patients with CAA-related ICH is secondary to the underlying CAA pathology rather than hypertension-related arteriosclerosis. We also found that the incidence of DWI lesions is not constant over time; it peaks shortly after the ICH (especially within three months) and is later followed by a lower incidence. Over the period of one year the estimated incidence of acute ischaemic infarction might reach 10 per person affected by CAA-related ICH. Our main limitation in this study was the robustness of the diagnosis of CAA as pathological confirmation was not available. In addition, there was possible selection bias in obtaining the MRI scans, although our analyses demonstrate that patients excluded from analysis were similar to patients included in terms of ICH severity (ICH volume) and GCS on admission. The median ICH volume and GCS scores were comparable to previously published data on symptomatic ICH from population-based studies (van Beijnum et al., 2009), thereby suggesting that our population was representative in terms of ICH severity. Our findings suggest that subclinical ischaemic lesions detected on MRI are a marker of the presence of an active underlying vasculopathy (CAA) leading to both ischaemia and haemorrhage. DWI positive lesions could be used as a surrogate marker with value in diagnosing or assessing the severity and progression of the disease.

9.1.2 Critical appraisal of our studies

Below we propose a critical appraisal of our studies against a set of criteria for ideal study design of CMBs (Cordonnier et al, 2007) and based on the recommendations for CMB mapping in research studies (Greenberg et al., 2009) (Table 30). These criteria were published following the principles of the Cochrane Collaboration, Quality Assessment of Diagnostic Accuracy Studies (QUADAS) (Whiting et al., 2003).

Based on the ideal study design for CMBs, our studies did not assess CMBs as predictors of prognosis. Such objective would require prospective study designs, with predefined and validated outcome measures, outcomes assessed blind to prognostic factors of interest, stratification of outcome by differences in treatment and satisfactory levels of follow-up (Cordonnier et al., 2007). Our studies were studies of the association of CMBs with clinical and/or radiological features (except for the study on the reliability of the MARS scale, chapter 4). They also evaluated the prevalence of CMBs in particular disease groups (hospital-based population, the majority of patients were white Caucasians, with ischaemic stroke/TIA or ICH cohort). As such, they could not assess causality in the relationship studied. According to these principles, such studies should include a specification of MRI parameters, a suitable GRE 2* sequence, a clear definition of CMBs size and appearance, a clear definition of participtants clinical and radiological diagnosis, independent of risk factors (Table 29). Other desirable study attributes are generalizability of the population and findings, exempt of selection bias. When assessing CMBs' association with other radiological features or clinical findings, an appropriate comparison group should be chosen. Each group should have clearly defined risk factors and assessment of CMBs should be blinded to clinical features of interest (Cordonnier et al., 2007). Depending on the question being addressed (mainly assessment of risk or assessment of association), the ideal studies on CMBs will include the above characteristics to ensure adequate reporting of CMBs, minimise selection bias and ensure generalizability of the findings. In this thesis, our main limitations related to the use of retrospective study designs and small sample sizes, limiting the power of our analyses of associations between CMBs and clinical and radiological characteristics. We were only able to provide a limited assessment of validity and reliability of regression analyses, which were limited because of small sample sizes.

Table 30. **Critical appraisal of our studies against a set of criteria for ideal study design of CMBs**

Studies	Study aim	Clear specification of MRI parameters	Clear definition of CMBs	Participants diagnosis clearly defined	Participants generalizable	Risk factors clearly defined	Assessment of CMBs blinded to clinical and radiological data	Comparison group(s) chosen appropriately
Chapter 5. CMB and the risk of ICH in patients on anti-platelet treatment	Prevalence of CMBs in the ICH population and association of CMBs with clinical/radiological features	**√**	**√**	**√**	**No** Small sample size and potential selection bias: 1/ Non Asian cohort 2/ Possible high prevalence of CAA in ICH groups 3/ Selection of less severe ICH cases	**√** (age, sex, HTN, WML)	**Not fully** (readers were not blind to the presence of ICH, the severity of the WML, the pt's age)	**No** 1/Controls were not truly controls 2/There were no controls with neither ICH nor aspirin use 3/Cases not perfectly matched for HTN and previous history of stroke
Chapter 6. Exploration of CMB dynamics	Prevalence of CMBs in an IS/TIA population and association of CMBs with clinical/radiological features	**√**	**√**	**√**	**No** 1/ small sample size 2/ Symptomatic IS only 3/ Overrepresentation of deep ICH in patients who died	**√** (systolic BP at baseline, baseline CMB)	**Not fully** (readers were not blind to the severity of WML in some patients and to pt's age)	**√** but groups were not entirely comparable for hypertension, history of previous stroke and WML
Chapter 7.1. CMBs are associated with executive impairment	Prevalence of CMBs in a population with IS/TIA and association of CMBs with clinical/radiological features	**√**	**√**	**√**	**No** Large cohort but with selection bias: 1/ very severe strokes were excluded 2/ patients with severe dementia were excluded	**√** (age, HTN, WML) but other factors not measured (e.g. volume loss)	**Not fully** (readers not blind to the severity of WML in some patients, and to pt's age)	**√** but groups not entirely comparable in several characteristics (gender, HTN, diabetes, previous hx of stroke/TIA, use of antithrombotics, prevalence of lacunes and WML)
Chapter 7.2. Strictly lobar CMBs are associated with long term executive impairment	Prevalence of CMBs in a population with IS/TIA and association of CMBs with clinical/radiological features	**√**	**√**	**√**	**No** 1/ small sample size 2/ subsample of original study with significant drop out	**No** (not able to fully assess risk factors due to small sample size)	**Not fully** (readers not blind to severity of WML in some patients and to pts' age)	**√** (but differences in age, gender, HTN, previous history of stroke and premorbid intellectual functioning although differences not significant)

Chapter 8. Lobar CMBs are associated with acute silent ischaemia	Prevalence of CMBs in a population with ICH and association of CMBs with clinical/radiological features	**√**	**√**	**√**	No 1/ only symptomatic ICH were included 2/ most severe ICH cases were excluded 3/relatively small sample	**√** (age, sex, WML and strictly lobar CMBs), but we cannot exclude an effect of HTN, and ICH on ischaemia)	**Not fully** (readers not blind to presence and location of ICH, patient's age, and WML burden)	**√** but populations and imaging characteristics were slightly heterogeneous

9.1.3 Remaining questions and areas for future research

Can cerebral microbleeds be reliably mapped in the brain?

Rating and mapping CMBs is a challenge which we have attempted to provide practical solutions to through the development of a new scale (MARS) and recommendations for rating and mapping CMBs in research studies that have been incorporated in the scale (see Chapter 4). As discussed, CMB prevalence in research studies is influenced by the rating and imaging methodologies, and these should be systematically reported. Future studies should follow the standards for study design and recommendations for reporting of CMBs (Cordonnier et al., 2007; Greenberg et al., 2009b) in order to allow comparability and data pooling between studies. These include the use of a standardised rating instrument, and MARS could be used for this purpose. For full recommendations, the guidelines of the CMB study group are reported in Table 29 (Greenberg et al., 2009b). As a marker of bleeding prone angiopathies, CMBs are closely associated with other markers of small vessel diseases, and the reporting of these should follow the recently published STRIVE standards (Wardlaw et al., 2013).

Table 31. Recommendations for CMB mapping in research studies
from Greenberg et al., 2009

- Use standardised MRI parameters (field strength, spatial resolution, slice thickness, TE, post-processing, etc.) – especially relevant if studies are carried out in multiple centres
- Apply clear definition of CMBs and mimics – rigid size criteria are probably not needed
- Use a standardised rating instrument with clearly defined anatomical regions, appropriate to the research question (e.g. deep vs. lobar, or individual lobes)
- Scale should have good inter- and intra-rater reliability applied to sequences and used by the observers in the study
- Use trained observers (ideally a single observer for all analysis in a study)
- Observers should be blinded to clinical details relevant to the study hypothesis
- Images rated on diagnostic quality workstations in semi-dark conditions

To the best of our knowledge, no other rating scales of CMBs have been proposed to date other than those reported in this thesis, MARS and BOMBs. We recommend using MARS over BOMBs because MARS has marginally better reliability, and its anatomical categorisation of CMBs into lobes better reflects the potential clinical relevance of CMBs and their effect on brain function depending on the question being addressed and the population studied. For example, patients with CAA have CMBs predominantly in the

posterior lobar regions (temporal and occipital lobes) (Ayaz et al., 2010; Rosand et al., 2005), whilst patients with AD have a predominance of cortico-subcortical CMBs with occipital lobes accounting for 57% of these CMBs (Pettersen et al., 2008). However, these manual rating scales, although simple, reliable and inexpensive, are very time consuming and operator-dependent. Although they produce reliable and reproducible information, they may not be practical in research settings outside routine clinical practice.

Alternative imaging methods to GRE T2* MRI for CMB detection are currently under evaluation and should ultimately help to better depict and quantify CMBs. Some of these techniques, e.g. SWI, are currently being implemented in routine clinical settings, marking substantial progress in the routine diagnosis of brain vascular diseases. SWI increases the tissue contrast of CMBs (Sehgal et al., 2005), can detect smaller lesions (Schrag et al., 2010) and reveals more CMBs than conventional T2* GRE (Nandigam et al., 2009). In line with this, it was recently shown that SWI confers greater reliability and sensitivity in CMB detection than GRE T2* MRI (Cheng et al., 2013). SWI creates more complex images due to high sensitivity to other iron-containing structures (Mittal et al., 2009), and for example, reveals many flow voids from small blood vessels. However, in the study by Mittal et al. (2009), the increase in sensitivity of SWI did not come at the expense of a reduction of inter-rater reliability. In contrast with our study, there was a significant effect of rater experience in the sensitivity and reliability of SWI for CMB detection. This suggests that appropriate training is paramount when using sophisticated sequences. Similar to manual ratings using GRE T2*, the authors found that reliabilities were affected by the number of CMBs, as larger numbers are more time consuming and represent a challenge to the rater. Studies of populations with relatively large numbers of CMBs may have reduced rating reliability, which may affect CMB prevalence in these studies. The increased sensitivity and reliability of SWI in this study suggests that SWI might be better in these populations (Cheng et al., 2013). Further research is needed to establish whether SWI should be the best standard for CMB detection in research and clinical practice.

Further methods for detecting and measuring CMBs are in development. Similar to fully automated evaluations for white/grey matter and white matter lesion volumes (de Boer et al., 2009), automated methods for other measures such as CMBs are under investigation (Seghier et al., 2011). Automated methods represent a new opportunity for quickly and reliably mapping CMBs by detecting various properties of CMBs (e.g. signal characteristics, size, shape) in order to distinguish them from other tissue types. They potentially have

major benefits in terms of reliability and speed: they are quick, reliable, not operator dependent, and aim to reduce processing time and increase consistency over manual rating methods (Gregoire and Werring, 2011). They may allow the comparison of CMB anatomical distribution between patients groups using group overlap or probabilistic maps in standard stereotactic space, and also the automated quantification of CMB size and volume. However, the drawbacks of these methods are the difficulty of distinguishing mimics – with similar signal and morphological characteristics – from true CMBs, resulting in a relatively high prevalence of false positives, and the need for some observer intervention and research-quality rather than standard clinical scans.

Reports on automated methods have mainly used standard MR images (Seghier et al., 2011) but post-processing techniques (SWI) were recently proposed (Barnes et al., 2011). The technique with SWI relies on a statistical thresholding algorithm to identify hypointensities (Barnes et al., 2011), but its sensitivity appears lower than that of the standard manual review and consensus agreement. Therefore, for the moment we recommend using visual rating scales as they offer a simple and practical solution to assess the presence, number and distribution of brain CMBs on standard clinical-quality brain MR images without the need for sophisticated post-processing methods.

Ultimately, the most appropriate type of rating scale used will depend upon the particular clinical question(s) being addressed (for further reading, see Chapter 4, and Gregoire and Werring, 2011). For example, some studies may need to consider CMBs in specific arterial territories (e.g. investigations of arterial recanalisation interventions), whilst others may require classification into the cerebral lobes (e.g. cognitive correlations), and some will concentrate on deep versus lobar distributions of CMBs (e.g. diagnosis of CAA and other small vessel arteriopathies). The use of standardised rating scales in CMB studies should allow more informative cross-study comparisons, and if the rating method is fully standardised then this may also help to identify other sources of variation in CMB evaluation, including MRI acquisition strategies. Standardised rating instruments and CMB definition criteria will be essential in determining the value of newer MRI acquisitions techniques including SWI. It seems reasonable that CMB research studies wherever possible should use a standardised rating system with central rating by a single observer. These, and as many other study factors as possible, should be kept constant when longitudinal CMB data are collected in prospective studies. In the coming years more sophisticated automated or semi-quantitative methods for detecting CMBs are likely to

emerge, but there will remain a need for simple, practical rating scales that can be applied to routinely-collected clinical datasets.

What is the etiology of cerebral microbleeds?

To date, the exact etiology of CMBs remains unclear. Histopathological data of CMBs remains scarce which limits our understanding of their potential causes. Most studies have included the MRI analysis of living patients and a separate histopathological analysis of post-mortem brains. Only a few studies have conducted an analysis of pre- and post-mortem brains and the number of brains studied was small. Nevertheless, CMBs have usually been attributed as resulting from an increased vascular fragility in patients with various types of small vessel disease. Theoretically, CMBs may be caused by the steady extravasation of erythrocytes through the damaged vessel walls of small brain arteries. An increased vascular fragility of the vessel wall due to chronic lipofibrohyalinosis or CAA causes their rupture. It is possible that these underlying microangiopathies lead to the formation of the microaneurysms or pseudoaneurysmal lesions that were originally described by Charcot and Bouchard. However, we do not yet know how these lesions relate to CMBs and their clinical significance is remains to be verified.

There is insufficient data from the current literature to tell us whether CMBs are a primary event, an ICH at a very small scale, or whether they represent a general marker of increased small vessel fragility. In keeping with the 'bleeding' hypothesis, it is possible that they develop from tiny areas of frank haemorrhage in a similar way to large spontaneous haematomas. If this is the case, then the factors preventing further extension of the bleeding to cause larger haematomas remain unknown. Certain circumstances (e.g. the use of antithrombotics) may play a role and some facts come to support this hypothesis: (1) incident symptomatic ICHs have been reported at the site of a previous CMB (Fan et al., 2003; Huang et al., 2008); and (2) patients with CMBs have been found to have a similar pattern of vasculopathy to those with ICH (Rosand et al., 2005; Ayaz et al., 2010). The association of age-related cerebrovascular ischaemic disease and lobar CAA-related and deep hypertensive haemorrhages suggests the occurrence of small haemorrhages in the development of CMBs. However, some facts from CAA patients suggest that CMBs and ICH represent distinct pathophysiological events: (1) the volumes of the haemorrhagic lesions fall into two distinct peaks of CMBs and macrobleeds, rather than forming a single continuous distribution; and (2) CMBs are associated with increased vessel thickness in amyloid-laden vessels, which is not observed in areas with macrobleeds and fewer CMBs

(Greenberg et al., 2009a). These findings suggest that particular properties of the diseased vessel may predispose to the formation of CMBs rather than macrobleeds or vice versa, and that lobar CMBs and lobar ICH represent independent vascular rupture events (Khan et al., 2011).

Possible explanations of the mechanisms leading to the formation of CMBs come from the close interplay between the ischaemic and haemorrhagic processes associated with the underlying vasculopathies. There is a preliminary but important indication that CMBs detected as a hypointense focus on T2*-GRE weighted imaging may pathologically correspond to a more diverse spectrum of pathology, including microhaemorrhages and microinfarcts (Janaway et al., 2013; Shoamanesh et al., 2013). After ICH some lesions exhibiting restricted diffusion on DWI MRI have imaging characteristics that can be attributed to either infarct or haemorrhage (Shoamanesh et al., 2013). We found a high prevalence of DWI positive lesions in patients who recently sustained a CAA-related ICH, suggesting the presence of active ischaemia (Gregoire et al., 2011). Further reports have confirmed that incident microinfarcts and microhaemorrhages occur concurrently after ICH (Menon et al., 2012; Shoamanesh et al., 2013). A sequence of events has been hypothesised to explain this phenomenon in the setting of a diseased microvasculature, one of which leads to the formation of CMBs. Firstly, the increasing intracranial BP results in a rise in systemic BP that causes the rupture of remote fragile vessels and CMBs; then, aggressive BP lowering causes a haemodynamic insufficiency which the diseased microvasculature cannot compensate, resulting in the development of microischaemic infarcts (Menon et al., 2012; Shoamanesh et al., 2013). These data suggest that after ICH, and possibly IS, for reasons that are still unknown, the activity of the cerebral small vessel diseases flares up, resulting in new microischaemic and microhaemorrhagic lesions. CMBs are one of the manifestations of the close interplay between the active ischaemic and haemorrhagic processes occurring in cerebral vasculopathies.

Recent data challenges the hypothesis that CMBs result from microbleeding from diseased vessels. The hypothesis made by the group led by Paul Ince is that haemosiderin deposits in the brain come from local iron sources that are released after ischaemia (Janaway et al., 2013). The ischaemic processes caused by small vessel diseases causes a release of the iron stores which exceeds the ability of the tissues, especially ageing tissues, to process it. In elderly patients with small vessel disease, the excess of iron accumulates in perivascular macrophages, which are seen in histopathological studies. CMBs may therefore be a

marker of focal haemosiderin deposition secondary to parenchymal ischaemic damage, rather than the result of microbleeding. In their histopathological study, most patients had haemosiderin deposits, suggesting that standard MR techniques underestimate the presence of CMBs. These findings will need to be confirmed in other studies.

Advances in the understanding of CAA have the promise to determine where CMBs fit in the pathogenesis of the disease and elucidate the mechanisms leading to their formation. New non-invasive techniques capable of detecting amyloid deposition in the brain *in vivo*, e.g. Positron Emission Tomography (PET) imaging with amyloid Pittsburgh compound B (PiB) have been developed (Johnson et al., 2007; Klunk et al., 2004; Mathis et al., 2003). This agent has the ability to detect CAA before it causes symptomatic ICH or CMBs (Charidimou et al., 2012b), which might provide useful insights into CMB formation. A recent study using PET-PiB in clinically probable CAA found that CMBs correspond to areas with a high concentration of amyloid, e.g. lobar (Dierksen et al., 2010), and PiB retention declined with increasing distance from the CMBs (Johnson et al., 2007). PET-PiB also provides useful insights in the pathogenesis of deep and lobar CMBs. In a recent study of patients with AD and subcortical VCI, it was shown that subcortical small vessel disease and amyloid-related pathologies (e.g. CAA) both contribute to their pathogenesis with a synergistic effect (Park et al., 2013). Other recently introduced techniques, such as SWI, a three-dimensional T2*-GRE technique, enables visualisation of CMBs with much increased sensitivity, resulting in higher lesion counts (at least 67% more compared with conventional T2*-GRE) with potential implications in the understanding of their pathogenesis (Nandigam et al., 2009). Further recent developments in the field of high resolution MR imaging enable the detection of small vessels *in vivo*, and should shed light into the mechanisms of CMBs, but these techniques are yet to be integrated into clinical practice (Harb et al., 2013).

A full understanding of the pathogenesis of CMBs will require a large prospective cohort study of patients with ischaemic and haemorrhagic small vessel diseases, compared with age-matched controls, and combine longitudinal clinical and multimodal radiological data. Pre-mortem and post-mortem brain examination correlated with post-mortem brain scans will be needed to allow comparisons and correlation with vascular risk factors, demographic and radiological characteristics using validated tools. The feasibility of such a study is limited, which explains why numerous questions on the etiology of CMBs remain incompletely answered.

Temporal changes of cerebral microbleeds: are they static or dynamic?

From studies demonstrating the temporal changes of CMBs (Chen et al., 2006; Goos et al., 2010; Greenberg et al., 1999; Orken et al., 2013; Poels et al., 2011), it seems clear that they are a dynamically changing phenomenon rather than a static marker of small vessel disease. CMBs present a rapid evolution within the first few days after an acute IS, suggesting a widespread small pathological process associated with acute ischaemia. In addition, they generally do not disappear over time (Poels et al., 2011), and our findings also support the idea that CMBs are a dynamic process (Gregoire et al., 2010a). We demonstrated that one in two patients with previous IS or TIA develops new CMBs in the following five years after their stroke/TIA (Gregoire et al., 2010a). The main risk factor associated with new CMB formation was a high baseline BP. The potential benefit of more aggressive BP lowering is currently being tested in the PRESERVE trial. Further research is needed to determine the temporal changes of CMBs over longer term periods and to understand the long-term clinical implications of acute CMB development after acute ischaemia and ICH, with potential implications for treatment. There is already good reason to treat hypertension and other risk factors aggressively in small vessel disease but CMBs may strengthen the case.

Are cerebral microbleeds useful diagnostic markers of brain small vessel diseases?

There is no doubt from neuroimaging and neuropathological studies that CMBs are a marker of CAA, hypertensive small vessel disease and other small vessel diseases. Histopathological correlation has shown that CMBs occur in association with small vessel diseases (Fazekas et al., 1999). In CAA, the sensitivity of the Boston criteria increased when CMBs were included as haemorrhagic lesions (Charidimou et al., 2012b), and an increasing body of evidence suggests that the spatial distribution of CMBs may allow the differential diagnosis of the underlying bleeding-prone arteriopathy. We have indirect (but not proven) evidence that CMBs in an exclusively deep distribution result from hypertensive arteriopathy, whilst those at the cortico-subcortical boundaries of the cerebral lobes are related to CAA (Poels et al., 2010). Lines of evidence supporting this hypothesis come from: (1) the study of the genetic polymorphisms associated with CMBs suggesting that strictly lobar CMBs are an imaging biomarker of CAA (Maxwell et al., 2011); (2) neuroimaging studies in clinically probable CAA using PiB-PET showing that CMBs correspond to areas

with a high concentration of amyloid (Dierksen et al., 2010); and (3) neuroimaging studies of CMBs in patients with ICH, where patients with hypertensive ICH are more likely to have deep CMBs, whereas patients with lobar ICH tend to have strictly lobar CMBs (Lee et al., 2004c; Roob et al., 2000; Smith et al., 2010). However, this dichotomic distinction between deep and lobar CMBs has limitations, as hypertensive arteriopathy (lipofibrohyalinosis) has been shown to affect superficial perforating vessels arising from pial arteries (Werring, 2011), whereas conversely, amyloid deposits have been detected in deep regions in AD (Ogomori et al., 1989).

If their spatial distribution can be mapped, CMBs may be more specific for the underlying pathology than some other imaging manifestations of small vessel diseases (e.g. WML). More sensitive imaging techniques using 'high-end' sequences (e.g. higher field strength, higher resolution, or use of advanced post-processing) compared with conventional T2*-weighted imaging, may contribute to more accurately identifying persons with underlying small-vessel pathology, with potential relevance for subsequent management (Poels et al., 2012b). Studies using PET-PiB may provide new data concerning the associations between deep and lobar CMBs and the underlying small vessel processes. A recent study using PET-PiB and MRI in patients with cognitive impairment showed that WML and lacunes contribute to both deep and lobar CMBs, whereas amyloid deposition was associated with the likelihood of having lobar (but not deep) CMBs (Park et al., 2013). Thus, CMBs may have the promise to detect and quantify the effects of small vessel disease and amyloid deposition.

Are cerebral microbleeds a prognostic marker of increased future stroke risk?

There is increasing evidence that CMB formation contributes to new clinical events and a higher stroke risk in stroke patients:

(1) Their contribution to transient neurological disturbance has been mostly studied in cross-sectional studies (Greenberg et al., 1993; Roch et al., 2005) and in a meta-analysis (Charidimou et al., 2012a). We described two cases of neurological dysfunction in patients with acute symptomatic CMBs, but these are not reported in this thesis because of space limitations (James et al., 2011)

(2) A recent meta-analysis suggested that CMBs increase the risk of any recurrent stroke, especially ICH (O.R 8.52, 95%CI 4.23-17.18) and to a lesser extent IS (OR 1.55, 95%CI 1.12-

2.13) (Charidimou et al., 2013a). This recent meta-analysis confirms the hypothesis of an increased stroke risk associated with CMBs that was previously suggested in small longitudinal and prospective studies. This risk varies among ethnic groups, with a greater risk of ICH among Asian populations, and a greater risk of recurrent IS in Western populations (Charidimou et al., 2013a). CMB distribution may also influence stroke risk, as lobar CMBs are a stronger risk factor for ICH than deep CMBs. More data are required to assess the effect of CMB distribution on stroke risk.

Large prospective data for patients with CMBs compared to CMB-free controls and testing their prognostic relevance for the risk of new clinical events (including transient neurological events, subsequent TIA, IS and ICH), cognitive decline and mortality, are required to confirm these findings. We need to understand the influence of CMB burden and distribution, and crucially whether CMBs affect the future balance of risk between ICH and IS. Large meta-analyses will be essential to elucidating these aspects.

Do cerebral microbleeds contribute to cognitive impairment?

Cross-sectional and small longitudinal studies suggest that CMBs impact on executive cognitive functions in stroke patients (Gregoire et al., 2013; Werring et al., 2004). We found a strong independent association between CMBs and executive impairment in stroke patients that persist over time (Gregoire et al., 2012) and that is largely driven by CMBs in a strictly lobar distribution (Gregoire et al., 2013) (see Chapter 7). These findings are consistent with the results of other studies showing a direct contribution of CAA-related CMBs to cognitive impairment (Ayaz et al., 2010; Viswanathan et al., 2010). Although these results need to be confirmed in larger longitudinal and prospective studies, they suggest an independent role for CMB-associated vasculopathy in cognitive impairment.

The mechanism(s) by which CMBs might influence cognitive function remain speculative. They may reflect focal damage of brain tissue and may interfere with cognitive processes when located in strategic areas. A more likely explanation is that they are a more general marker for the severity of small vessel disease, and as such may influence cognition (Werring et al., 2010). This last hypothesis is of significant interest as it may help to unravel the potential key link between vascular and neurodegenerative pathologies (Cordonnier and van der Flier, 2011). Vascular damage induced by Aβ has been suggested to be a key step in the pathogenesis of AD (Ferrarese and Piazza, 2012). In patients with cognitive impairment due to subcortical small vessel disease or CAA, small vessel disease and

amyloid burden interactively affect cognitive functions (Park et al., 2013). Such an interaction between vascular disease and amyloid was demonstrated experimentally and suggests a magnifying effect of amyloid on ischaemia (Hachinski, 2011; Whitehead et al., 2007). In clinical trials of immune therapies directed against Aβ for the treatment of AD (Piazza et al., 2013; Sperling et al., 2012), some patients developed complications resulting from the anti-amyloid drugs characterised by amyloid-related imaging abnormalities (ARIA) on MRI. These MRI abnormalities were similar to those of CAA-related inflammation (CAA-ri) and seem to be immune-mediated (Piazza et al., 2013). Further trials of Aβ immunotherapy correlating anti-Aβ dosage to MRI data (and identification of amyloid *in vivo* using PET- PiB) will help to better understand the pathogenic mechanisms linking CAA and AD, and the role that CMBs play in the development of cognitive impairment in these patients.

Are antiplatelets and anticoagulants safe in patients with cerebral microbleeds?

The limited prospective data are consistent with the hypothesis that CMBs increase the risk of ICH as a complication of antithrombotic use. On the other hand, CMBs are also associated with recurrent IS, creating a clinical dilemma concerning the use of antithrombotic drugs in patients with CMBs. In certain population groups (e.g. patients with a high vascular risk and numerous lobar CMBs, and patients diagnosed with CAA), the risk/benefit ratio of antithrombotic drugs may shift towards an increased risk of ICH. We found an independent association between CMBs and ICH in patients on anti-platelet treatment in a small case-control study (see Chapter 5). We observed a predominance of lobar ICH and lobar CMBs in the antiplatelet and non-antiplatelet users, suggesting a possible overrepresentation of patients with CAA compared with other studies. Nevertheless, clinicians should cautiously weigh the risks and benefits of antiplatelet treatment in individuals diagnosed with CAA, especially since recurrent ICH with aspirin use in CAA appears to be moderated by CMB burden. Further studies evaluating the relative effects of CMB burden on future ICH and IS risk are warranted to determine whether a certain CMB load might tip the risk-benefit balance in favour of avoiding antithrombotic treatment (Charidimou et al., 2013a). Although data continues to accumulate, no definite answer has yet emerged.

Several key questions regarding the use of antithrombotic drugs in patients with CMBs remain unanswered, including the underlying pathophysiology mechanisms for the associations between antithrombotic agents with CMBs, the possible cause-effect link

between antithrombotic agents and CMBs, the relationship between dosage and duration and the prevalence and lesion load of CMBs, the risks specific to different ethnic groups, and whether the risk is related to a specific distribution of CMBs. CAA, characterised by the presence of strictly lobar CMBs, is increasingly recognised as a cause of anticoagulant-related ICH (Greenberg et al., 2009b). Therefore, we need more data on CMB distribution in studies evaluating the impact of antithrombotic agents in ICH.

With regard to anticoagulant use, there are no large prospective studies of CMBs in IS cohorts treated with anticoagulants for AF. We only have indirect evidence of an association between lobar CMBs, reflecting CAA and the risk of anticoagulant-related ICH (Charidimou et al., 2012b; Rosand et al., 2000). Future studies will need to prospectively follow patients anticoagulated after IS associated with AF and we look forward to the results of the CROMIS-2 trial.

9.1.4 Implications for future clinical trials

In the coming years more prospective data from large clinical trials will contribute to answering the remaining questions on CMBs. The majority of current knowledge is based on data that was cross-sectionally acquired, precluding the understanding of cause and effect relationships between CMBs and clinical events. Large cohorts will be needed to study the genetic contribution to the development of CMBs and ICH in stroke patients. In the analysis of their observations, researchers will need to prioritise the use of continuous over dichotomous outcomes, as continuous data provides more sensitive and powerful analyses. Studies will also need to ensure that raters are blinded to all clinical data. In studies evaluating the impact of CMBs on cognitive functions, repeated cognitive screening is required and analyses will need to take into account possible learning effects. With regard to the critical question of the safety of antithrombotic drugs in patients with CMBs, valid prospective data is required to determine the causes, incidence and risk factors of CMBs and ICH in bleeding-prone populations (e.g. with numerous lobar CMBs and in the presence of previous ICH). Stratification of populations and CMB carriers according to ethnicity, specific agent and duration of therapy will inform the safety of use of antithrombotic agents, and risk-benefit analyses will allow physicians to make informed decisions. These studies will need to ensure that risk factors (e.g. BP) are adequately controlled for as this may influence outcomes. Long follow-up times in prospective studies will be needed to reliably investigate the relationship between CMBs and clinical outcomes. The standardised use of imaging methods and rating scales will facilitate the pooling of

data from different centres and studies, allowing an optimised study of their clinical effects in large cohorts. This will be especially important in studying the risk of anti-thrombotic related ICH associated with CMBs, as the number of clinical events remains small and the population size required to study such events is large. Because of the strong association between CMBs and other markers of cerebrovascular disease, bias should be minimised by adjusting for all potential confounders that may influence the associations tested. In particular, the possible association between CMBs and antithrombotic drugs is potentially confounded by the indications for which the drugs are prescribed: antithrombotics are more likely to be prescribed to patients at higher risk of developing CMBs; and there is a strong relationship between CMBs and cerebrovascular diseases which is independent of the use of antithrombotic agents. Therefore, future studies addressing the question of the safety of antithrombotic agents in patients with CMBs will require a strict matching process in homogeneous populations.

9.2 Main conclusions

The discovery of CMBs over the past decade has revealed that presymptomatic small vessel pathology due to chronically increased BP or CAA is a factor associated with an increased risk of future symptomatic cerebrovascular disease, including larger haematomas, IS and cognitive impairment. Nowadays, following substantial advances in imaging techniques, areas of signal abnormalities in the brain, including silent WML and CMBs, have allowed the evidence of the underlying small vessel changes that were previously only visible at autopsy to be highlighted (see Chapters 1 and 2). As a surrogate marker of the underlying bleeding-prone microangiopathies, these abnormalities have generated significant clinical interest as potential predictors of future neurological decline and vascular risk (see Chapter 3). Because these microangiopathies are associated with an increased ICH risk, CMBs have generated increasing interest as potential predictors of ICH, especially related to the use of antithrombotics. CMBs may have potential in helping to assess the risk-benefit ratio of administering antithrombotic drugs following a stroke. A growing literature, including our work, shows that they have numerous other clinical implications including a role in recurrent IS, cognitive decline, disability and death.

CMBs are a marker of vascular vulnerability secondary to age-related microvessel changes, including lipohyalinosis and CAA. Questions remain on the mechanisms leading to their formation which should be answerable through studies using the emerging detection techniques (e.g. PET-PiB, SWI), ideally correlated with results from definite pathology. These new techniques will shed light on the pathogenesis of the underlying microangiopathies. Ultimately, the early detection of small vessel diseases may allow more effective interventions to reduce the burden of stroke, cognitive impairment and other major challenges facing our ageing societies.

References

Adams, H. P., Jr., Bendixen, B. H., Kappelle, L. J., Biller, J., Love, B. B., Gordon, D. L. & Marsh, E. E., 3rd 1993. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke,* 24**,** 35-41.

Aharon-Peretz, J., Cummings, J. L. & Hill, M. A. 1988. Vascular dementia and dementia of the Alzheimer type. Cognition, ventricular size, and leuko-araiosis. *Arch Neurol,* 45**,** 719-21.

Algra, A., Gorter, J. W. & Kappelle, L. J. 1997. A randomized trial of anticoagulants versus aspirin after cerebral ischemia of presumed arterial origin. The Stroke Prevention in Reversible Ischemia Trial (SPIRIT) Study Group. *Ann Neurol,* 42**,** 857-65.

Allsop, D. 2000. Introduction to Alzheimer's disease. *In:* Hooper, N. (ed.) *Methods in molecular medicine, Vol. 32: Alzheimer's Disease: Methods and Protocols.* Totowas, NJ: Humana Press.

Almansori, M., Naik, S. & Ahmed, S. N. 2008. Magnetic susceptibility in a patient with a metallic heart valve. *Pak J Neurol Sci,* 3**,** 40-41.

Almkvist, O., Wahlund, L. O., Andersson-Lundman, G., Basun, H. & Backman, L. 1992. White-matter hyperintensity and neuropsychological functions in dementia and healthy aging. *Arch Neurol,* 49**,** 626-32.

Anderson, C. S., Huang, Y., Wang, J. G., Arima, H., Neal, B., Peng, B., Heeley, E., Skulina, C., Parsons, M. W., Kim, J. S., Tao, Q. L., Li, Y. C., Jiang, J. D., Tai, L. W., Zhang, J. L., Xu, E., Cheng, Y., Heritier, S., Morgenstern, L. B., Chalmers, J. & Investigators, I. 2008. Intensive blood pressure reduction in acute cerebral haemorrhage trial (INTERACT): a randomised pilot trial. *Lancet Neurol,* 7**,** 391-9.

Anon 1994. Risk factors for stroke and efficacy of antithrombotic therapy in atrial fibrillation. Analysis of pooled data from five randomized controlled trials. *Arch Intern Med,* 154**,** 1449-57.

Anon 1996. Bleeding during antithrombotic therapy in patients with atrial fibrillation. The Stroke Prevention in Atrial Fibrillation Investigators. *Arch Intern Med,* 156**,** 409-16.

Anon 2012. A united approach to vascular disease and neurodegeneration. *Lancet Neurol,* 11**,** 293.

Antithrombotic Trialists Collaboration 2002. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ,* 324**,** 71-86.

Antithrombotic Trialists Collaboration, Baigent, C., Blackwell, L., Collins, R., Emberson, J., Godwin, J., Peto, R., Buring, J., Hennekens, C., Kearney, P., Meade, T., Patrono, C., Roncaglioni, M. C. & Zanchetti, A. 2009. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. *Lancet,* 373**,** 1849-60.

Appelman, A. P., Exalto, L. G., van der Graaf, Y., Biessels, G. J., Mali, W. P. & Geerlings, M. I. 2009. White matter lesions and brain atrophy: more than shared risk factors? A systematic review. *Cerebrovasc Dis,* 28**,** 227-42.

Arboix, A. & Marti-Vilalta, J. L. 2009. Lacunar stroke. *Expert Rev Neurother,* 9**,** 179-96.

Aribisala, B. S., Valdes Hernandez, M. C., Royle, N. A., Morris, Z., Munoz Maniega, S., Bastin, M. E., Deary, I. J. & Wardlaw, J. M. 2013. Brain atrophy associations with white matter lesions in the ageing brain: the Lothian Birth Cohort 1936. *Eur Radiol,* 23**,** 1084-92.

Arima, H., Tzourio, C., Anderson, C., Woodward, M., Bousser, M. G., MacMahon, S., Neal, B., Chalmers, J. & Group, P. C. 2010. Effects of perindopril-based lowering of blood pressure on intracerebral hemorrhage related to amyloid angiopathy: the PROGRESS trial. *Stroke,* 41**,** 394-6.

Arvanitakis, Z., Leurgans, S. E., Wang, Z., Wilson, R. S., Bennett, D. A. & Schneider, J. A. 2011. Cerebral amyloid angiopathy pathology and cognitive domains in older persons. *Ann Neurol,* 69**,** 320-7.

Atlas, S. W., Mark, A. S., Grossman, R. I. & Gomori, J. M. 1988. Intracranial hemorrhage: gradient-echo MR imaging at 1.5 T. Comparison with spin-echo imaging and clinical applications. *Radiology,* 168**,** 803-7.

Attems, J. 2005. Sporadic cerebral amyloid angiopathy: pathology, clinical implications, and possible pathomechanisms. *Acta Neuropathol,* 110**,** 345-59.

Attems, J., Jellinger, K., Thal, D. R. & Van Nostrand, W. 2011. Review: sporadic cerebral amyloid angiopathy. *Neuropathol Appl Neurobiol,* 37**,** 75-93.

Au, R., Massaro, J. M., Wolf, P. A., Young, M. E., Beiser, A., Seshadri, S., D'Agostino, R. B. & DeCarli, C. 2006. Association of white matter hyperintensity volume with decreased cognitive functioning: the Framingham Heart Study. *Arch Neurol,* 63**,** 246-50.

Awad, I. A., Spetzler, R. F., Hodak, J. A., Awad, C. A. & Carey, R. 1986. Incidental subcortical lesions identified on magnetic resonance imaging in the elderly. I. Correlation with age and cerebrovascular risk factors. *Stroke,* 17**,** 1084-9.

Ayaz, M., Boikov, A. S., Haacke, E. M., Kido, D. K. & Kirsch, W. M. 2010. Imaging cerebral microbleeds using susceptibility weighted imaging: one step toward detecting vascular dementia. *J Magn Reson Imaging,* 31**,** 142-8.

Bae, H., Jeong, D., Doh, J., Lee, K., Yun, I. & Byun, B. 1999. Recurrence of bleeding in patients with hypertensive intracerebral hemorrhage. *Cerebrovasc Dis,* 9**,** 102-8.

Baezner, H., Blahak, C., Poggesi, A., Pantoni, L., Inzitari, D., Chabriat, H., Erkinjuntti, T., Fazekas, F., Ferro, J. M., Langhorne, P., O'Brien, J., Scheltens, P., Visser, M. C., Wahlund, L. O., Waldemar, G., Wallin, A., Hennerici, M. G. & Group, L. S. 2008. Association of gait and balance disorders with age-related white matter changes: the LADIS study. *Neurology,* 70**,** 935-42.

Ballard, C., Stephens, S., McLaren, A., Wesnes, K., Kenny, R. A., Burton, E., O'Brien, J. & Kalaria, R. 2002. Neuropsychological deficits in older stroke patients. *Ann N Y Acad Sci,* 977**,** 179-82.

Barba, R., Martinez-Espinosa, S., Rodriguez-Garcia, E., Pondal, M., Vivancos, J. & Del Ser, T. 2000. Poststroke dementia : clinical features and risk factors. *Stroke,* 31**,** 1494-501.

Barber, R., Scheltens, P., Gholkar, A., Ballard, C., McKeith, I., Ince, P., Perry, R. & O'Brien, J. 1999. White matter lesions on magnetic resonance imaging in dementia with Lewy bodies, Alzheimer's disease, vascular dementia, and normal aging. *J Neurol Neurosurg Psychiatry,* 67**,** 66-72.

Barnes, S. R., Haacke, E. M., Ayaz, M., Boikov, A. S., Kirsch, W. & Kido, D. 2011. Semiautomated detection of cerebral microbleeds in magnetic resonance images. *Magn Reson Imaging,* 29**,** 844-52.

Basile, A. M., Pantoni, L., Pracucci, G., Asplund, K., Chabriat, H., Erkinjuntti, T., Fazekas, F., Ferro, J. M., Hennerici, M., O'Brien, J., Scheltens, P., Visser, M. C., Wahlund, L. O., Waldemar, G., Wallin, A., Inzitari, D. & Group, L. S. 2006. Age, hypertension, and lacunar stroke are the major determinants of the severity of age-related white matter changes. The LADIS (Leukoaraiosis and Disability in the Elderly) Study. *Cerebrovasc Dis,* 21**,** 315-22.

Baumann, C. R., Schuknecht, B., Lo Russo, G., Cossu, M., Citterio, A., Andermann, F. & Siegel, A. M. 2006. Seizure outcome after resection of cavernous malformations is better when surrounding hemosiderin-stained brain also is removed. *Epilepsia,* 47**,** 563-6.

Beitzke, H. 1936. Die Rolle der kleinen aneurysmen bei den Massenblutungen des Gehirns. *Verh Dtsch Ges Pathol,* 29**,** 74-80.

Belokrenitzky, S. 1911. *De la dégénérescence amyloide des nerfs.,* Genève, Thèse de médecine.

Benisty, S., Gouw, A. A., Porcher, R., Madureira, S., Hernandez, K., Poggesi, A., van der Flier, W. M., Van Straaten, E. C., Verdelho, A., Ferro, J., Pantoni, L., Inzitari, D., Barkhof, F., Fazekas, F. & Chabriat, H. 2009. Location of lacunar infarcts correlates with cognition in a sample of non-disabled subjects with age-related white-matter changes: the LADIS study. *J Neurol Neurosurg Psychiatry,* 80**,** 478-83.

Benson, M. D., Skinner, M. & Cohen, A. S. 1977. Amyloid deposition in a renal transplant in familial Mediterranean fever. *Ann Intern Med,* 87**,** 31-4.

Biffi, A., Sonni, A., Anderson, C. D., Kissela, B., Jagiella, J. M., Schmidt, H., Jimenez-Conde, J., Hansen, B. M., Fernandez-Cadenas, I., Cortellini, L., Ayres, A., Schwab, K., Juchniewicz, K., Urbanik, A., Rost, N. S., Viswanathan, A., Seifert-Held, T., Stoegerer, E. M., Tomas, M., Rabionet, R., Estivill, X., Brown, D. L., Silliman, S. L., Selim, M., Worrall, B. B., Meschia, J. F., Montaner, J., Lindgren, A., Roquer, J., Schmidt, R., Greenberg, S. M., Slowik, A., Broderick, J. P., Woo, D., Rosand, J. & International Stroke Genetics, C. 2010a. Variants at APOE influence risk of deep and lobar intracerebral hemorrhage. *Ann Neurol,* 68**,** 934-43.

Biffi, A., Halpin, A., Towfighi, A., Gilson, A., Busl, K., Rost, N., Smith, E. E., Greenberg, M. S., Rosand, J. & Viswanathan, A. 2010b. Aspirin and recurrent intracerebral hemorrhage in cerebral amyloid angiopathy. *Neurology,* 75**,** 693-8.

Birns, J., Morris, R., Jarosz, J., Markus, H. S. & Kalra, L. 2009. Hypertension-related cognitive decline: is the time right for intervention studies? *Minerva Cardioangiol,* 57**,** 813-30.

Black, S., Gao, F. & Bilbao, J. 2009. Understanding white matter disease: imaging-pathological correlations in vascular cognitive impairment. *Stroke,* 40**,** S48-52.

Bokura, H., Kobayashi, S. & Yamaguchi, S. 1998. Distinguishing silent lacunar infarction from enlarged Virchow-Robin spaces: a magnetic resonance imaging and pathological study. *J Neurol,* 245**,** 116-22.

Boone, K. B., Miller, B. L., Lesser, I. M., Mehringer, C. M., Hill-Gutierrez, E., Goldberg, M. A. & Berman, N. G. 1992. Neuropsychological correlates of white-matter lesions in healthy elderly subjects. A threshold effect. *Arch Neurol,* 49**,** 549-54.

Boulanger, J. M., Coutts, S. B., Eliasziw, M., Gagnon, A. J., Simon, J. E., Subramaniam, S., Sohn, C. H., Scott, J., Demchuk, A. M. & Group, V. S. 2006. Cerebral microhemorrhages predict new disabling or fatal strokes in patients with acute ischemic stroke or transient ischemic attack. *Stroke,* 37**,** 911-4.

Bowler, J. V. 2005. Vascular cognitive impairment. *J Neurol Neurosurg Psychiatry,* 76 Suppl 5**,** v35-44.

Bowler, J. V. 2007. Modern concept of vascular cognitive impairment. *Br Med Bull,* 83**,** 291-305.

Brandner, S. 2011. Histopathology of cerebral microbleeds. *In:* Werring, D. J. (ed.) *Cerebral microbleeds: Pathophysiological to clinical practice.* Cambridge University Press.

Breteler, M. M. 2000. Vascular involvement in cognitive decline and dementia. Epidemiologic evidence from the Rotterdam Study and the Rotterdam Scan Study. *Ann N Y Acad Sci,* 903**,** 457-65.

Broderick, J., Connolly, S., Feldmann, E., Hanley, D., Kase, C., Krieger, D., Mayberg, M., Morgenstern, L., Ogilvy, C. S., Vespa, P., Zuccarello, M., American Heart, A., American Stroke Association Stroke, C., High Blood Pressure Research, C., Quality of, C. & Outcomes in Research Interdisciplinary Working, G. 2007. Guidelines for the management of spontaneous intracerebral hemorrhage in adults: 2007 update: a guideline from the American Heart Association/American Stroke Association Stroke Council, High Blood Pressure Research Council, and the Quality of Care and Outcomes in Research Interdisciplinary Working Group. *Stroke,* 38**,** 2001-23.

Bronge, L., Bogdanovic, N., Wahlund, L.O., et al. 2002. Postmortem MRI and histopathology of white matter changes in Alzheimer brains. A quantitative , comparative study. *Dement Geriatr Cogn Disord* , 13, 205-12.

Brott, T., Thalinger, K. & Hertzberg, V. 1986. Hypertension as a risk factor for spontaneous intracerebral hemorrhage. *Stroke,* 17**,** 1078-83.

Brown, J. J., Hesselink, J. R. & Rothrock, J. F. 1988. MR and CT of lacunar infarcts. *AJR Am J Roentgenol,* 151**,** 367-72.

Brown, M. A. & Semelka, R. C. 2003. *MRI: Basic principles and applications* New York, Wiley-Liss.

Brown, W. R., Moody, D. M., Thore, C. R. & Challa, V. R. 2000. Apoptosis in leukoaraiosis. *AJNR Am J Neuroradiol,* 21**,** 79-82.

Bruni, J., Bilbao, J. M. & Pritzker, K. P. 1977. Vascular amyloid in the aging central nervous system. Clinico-pathological study and literature review. *Can J Neurol Sci,* 4**,** 239-44.

Bryan, R. N., Cai, J., Burke, G., Hutchinson, R. G., Liao, D., Toole, J. F., Dagher, A. P. & Cooper, L. 1999. Prevalence and anatomic characteristics of infarct-like lesions on MR images of middle-aged adults: the atherosclerosis risk in communities study. *AJNR Am J Neuroradiol,* 20**,** 1273-80.

Burdette, J. H., Elster, A. D. & Ricci, P. E. 1998. Calculation of apparent diffusion coefficients (ADCs) in brain using two-point and six-point methods. *J Comput Assist Tomogr,* 22**,** 792-4.

Cadavid, D., Mena, H., Koeller, K. & Frommelt, R. A. 2000. Cerebral beta amyloid angiopathy is a risk factor for cerebral ischemic infarction. A case control study in human brain biopsies. *J Neuropathol Exp Neurol,* 59**,** 768-73.

Caggiati, A., Rosi, C., Franceschini, M. & Innocenzi, D. 2008. The nature of skin pigmentations in chronic venous insufficiency: a preliminary report. *Eur J Vasc Endovasc Surg,* 35**,** 111-8.

Carmichael, O., Schwarz, C., Drucker, D., Fletcher, E., Harvey, D., Beckett, L., Jack, C. R., Jr., Weiner, M., DeCarli, C. & Alzheimer's Disease Neuroimaging, I. 2010. Longitudinal changes in white matter disease and cognition in the first year of the Alzheimer disease neuroimaging initiative. *Arch Neurol,* 67**,** 1370-8.

Carrera, E. & Bogousslavsky, J. 2006. The thalamus and behavior: effects of anatomically distinct strokes. *Neurology,* 66**,** 1817-23.

Casanova, M. F. & Araque, J. M. 2003. Mineralization of the basal ganglia: implications for neuropsychiatry, pathology and neuroimaging. *Psychiatry Res,* 121**,** 59-87.

Chabriat, H., Vahedi, K., Iba-Zizen, M. T., Joutel, A., Nibbio, A., Nagy, T. G., Krebs, M. O., Julien, J., Dubois, B., Ducrocq, X. & et al. 1995. Clinical spectrum of CADASIL: a study of 7 families. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Lancet,* 346**,** 934-9.

Chabriat, H., Joutel, A., Dichgans, M., Tournier-Lasserve, E. & Bousser, M. G. 2009. Cadasil. *Lancet Neurol,* 8**,** 643-53.

Challa, V. R., Moody, D. M. & Bell, M. A. 1992. The Charcot-Bouchard aneurysm controversy: impact of a new histologic technique. *J Neuropathol Exp Neurol,* 51**,** 264-71.

Chamouard, J. M., Duyckaerts, C., Rancurel, G., Poisson, M. & Buge, A. 1988. [Transient ischemic attack in amyloid angiopathy]. *Rev Neurol (Paris),* 144**,** 598-602.

Chan, S., Kartha, K., Yoon, S. S., Desmond, D. W. & Hilal, S. K. 1996. Multifocal hypointense cerebral lesions on gradient-echo MR are associated with chronic hypertension. *AJNR Am J Neuroradiol,* 17**,** 1821-7.

Chao, C. P., Kotsenas, A. L. & Broderick, D. F. 2006. Cerebral amyloid angiopathy: CT and MR imaging findings. *Radiographics,* 26**,** 1517-31.

Charcot, J. M. & Bouchard, C. 1868. Nouvelles recherches sur la pathogénie de l'hémorragie cérébrale. *Arch Physiol Norm Pathol* 6425**,** 110-127.

Charidimou, A. & Werring, D. J. 2011. Cerebral microbleeds: detection, mechanisms and clinical challenges. *Future Neurology,* 6**,** 587-611.

Charidimou, A., Peeters, A., Fox, Z., Gregoire, S. M., Vandermeeren, Y., Laloux, P., Jager, H. R., Baron, J. C. & Werring, D. J. 2012a. Spectrum of transient focal neurological episodes in cerebral amyloid angiopathy: multicentre magnetic resonance imaging cohort study and meta-analysis. *Stroke,* 43**,** 2324-30.

Charidimou, A., Gang, Q. & Werring, D. J. 2012b. Sporadic cerebral amyloid angiopathy revisited: recent insights into pathophysiology and clinical spectrum. *J Neurol Neurosurg Psychiatry,* 83**,** 124-37.

Charidimou, A., Shakeshaft, C. & Werring, D. J. 2012c. Cerebral microbleeds on magnetic resonance imaging and anticoagulant-associated intracerebral hemorrhage risk. *Frontiers in Neurology* 3.

Charidimou, A., Kakar, P., Fox, Z. & Werring, D. J. 2013a. Cerebral microbleeds and recurrent stroke risk: systematic review and meta-analysis of prospective ischemic stroke and transient ischemic attack cohorts. *Stroke,* 44**,** 995-1001.

Charidimou, A., Krishnan, A., Werring, D. J. & Rolf Jager, H. 2013b. Cerebral microbleeds: a guide to detection and clinical relevance in different disease settings. *Neuroradiology,* 55**,** 655-74.

Charidimou, A., Kakar, P., Fox, Z. & Werring, D. J. 2013c. Cerebral microbleeds and the risk of intracerebral haemorrhage after thrombolysis for acute ischaemic stroke: systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry,* 84**,** 277-80.

Charletta, D., Gorelick, P. B., Dollear, T. J., Freels, S. & Harris, Y. 1995. CT and MRI findings among African-Americans with Alzheimer's disease, vascular dementia, and stroke without dementia. *Neurology,* 45**,** 1456-61.

Chen, Y. F., Chang, Y. Y., Liu, J. S., Lui, C. C., Kao, Y. F. & Lan, M. Y. 2008. Association between cerebral microbleeds and prior primary intracerebral hemorrhage in ischemic stroke patients. *Clin Neurol Neurosurg,* 110**,** 988-91.

Chen, Y. W., Gurol, M. E., Rosand, J., Viswanathan, A., Rakich, S. M., Groover, T. R., Greenberg, S. M. & Smith, E. E. 2006. Progression of white matter lesions and hemorrhages in cerebral amyloid angiopathy. *Neurology,* 67**,** 83-7.

Cheng, A. L., Batool, S., McCreary, C. R., Lauzon, M. L., Frayne, R., Goyal, M. & Smith, E. E. 2013. Susceptibility-Weighted Imaging is More Reliable Than T2*-Weighted Gradient-Recalled Echo MRI for Detecting Microbleeds. *Stroke,* 44**,** 2782-6.

Chimowitz, M. I., Estes, M. L., Furlan, A. J. & Awad, I. A. 1992. Further observations on the pathology of subcortical lesions identified on magnetic resonance imaging. *Arch Neurol,* 49**,** 747-52.

Cho, A. H., Lee, S. B., Han, S. J., Shon, Y. M., Yang, D. W. & Kim, B. S. 2009a. Impaired kidney function and cerebral microbleeds in patients with acute ischemic stroke. *Neurology,* 73**,** 1645-8.

Cho, H., Kwon, J. H. & Seo, H. J. 2009b. Medial temporal lobe atrophy in vascular dementia: visual temporal lobe rating scale. *Arch Gerontol Geriatr,* 48**,** 415-8.

Choi, P., Ren, M., Phan, T. G., Callisaya, M., Ly, J. V., Beare, R., Chong, W. & Srikanth, V. 2012. Silent infarcts and cerebral microbleeds modify the associations of white matter lesions with gait and postural stability: population-based study. *Stroke,* 43**,** 1505-10.

Cianchetti, F. A., Nishimura, N. & Schaffer, C. B. 2009. Cortical microhemorrhages reduce stimulus-evoked calcium responses in nearby neurons. *Journal of Cerebral Blood Flow and Metabolism* 29**,** S217-S218.

Cole, F. M. & Yates, P. O. 1967. The occurrence and significance of intracerebral micro-aneurysms. *J Pathol Bacteriol,* 93**,** 393-411.

Cole, F. M. & Yates, P. O. 1968. Comparative incidence of cerebrovascular lesions in normotensive and hypertensive patients. *Neurology,* 18**,** 255-9.

Compston, A. 2005. From the archives. 'Visual field changes following anterior temporal lobectomy: their significance in relation to "Meyer's loop" of the optic radiation' and 'The architecture of the optic radiation in the temporal lobe of man'. *Brain,* 128**,** 1959-61.

Conijn, M. M., Geerlings, M. I., Biessels, G. J., Takahara, T., Witkamp, T. D., Zwanenburg, J. J., Luijten, P. R. & Hendrikse, J. 2011. Cerebral microbleeds on MR imaging: comparison between 1.5 and 7T. *AJNR Am J Neuroradiol,* 32**,** 1043-9.

Copenhaver, B. R., Hsia, A. W., Merino, J. G., Burgess, R. E., Fifi, J. T., Davis, L., Warach, S. & Kidwell, C. S. 2008. Racial differences in microbleed prevalence in primary intracerebral hemorrhage. *Neurology,* 71**,** 1176-82.

Cordonnier, C., Klijn, C.J.M., van Beijnum, J., Al-Shahi Salman, R. 2010. Radiological investigation of spontaneous intracerebral hemorrhage: systematic review and trinational survey. *Stroke*, 41, 685-90.

Cordonnier, C., van der Flier, W. M., Sluimer, J. D., Leys, D., Barkhof, F. & Scheltens, P. 2006. Prevalence and severity of microbleeds in a memory clinic setting. *Neurology,* 66**,** 1356-60.

Cordonnier, C., Al-Shahi Salman, R. & Wardlaw, J. 2007. Spontaneous brain microbleeds: systematic review, subgroup analyses and standards for study design and reporting. *Brain,* 130**,** 1988-2003.

Cordonnier, C., Potter, G. M., Jackson, C. A., Doubal, F., Keir, S., Sudlow, C. L., Wardlaw, J. M. & Al-Shahi Salman, R. 2009. Improving interrater agreement about brain microbleeds: development of the Brain Observer MicroBleed Scale (BOMBS). *Stroke,* 40**,** 94-9.

Cordonnier, C. & van der Flier, W. M. 2011. Brain microbleeds and Alzheimer's disease: innocent observation or key player? *Brain,* 134**,** 335-44.

Corsellis, J. A. & Brierley, J. B. 1954. An unusual type of pre-senile dementia (atypical Alzheimer's disease with amyloid vascular change). *Brain,* 77**,** 571-87.

Cullen, K. M., Kocsi, Z. & Stone, J. 2005. Pericapillary haem-rich deposits: evidence for microhaemorrhages in aging human cerebral cortex. *J Cereb Blood Flow Metab,* 25**,** 1656-67.

Cummings, J. L. 1995. Anatomic and behavioral aspects of frontal-subcortical circuits. *Ann N Y Acad Sci,* 769**,** 1-13.

Davis, D. P., Robertson, T. & Imbesi, S. G. 2006. Diffusion-weighted magnetic resonance imaging versus computed tomography in the diagnosis of acute ischemic stroke. *J Emerg Med,* 31**,** 269-77.

de Boer, R., Vrooman, H. A., van der Lijn, F., Vernooij, M. W., Ikram, M. A., van der Lugt, A., Breteler, M. M. & Niessen, W. J. 2009. White matter lesion extension to automatic brain tissue segmentation on MRI. *Neuroimage,* 45**,** 1151-61.

de la Torre, J. C. 2002. Alzheimer disease as a vascular disorder: nosological evidence. *Stroke,* 33**,** 1152-62.

de Laat, K. F., Tuladhar, A. M., van Norden, A. G., Norris, D. G., Zwiers, M. P. & de Leeuw, F. E. 2011. Loss of white matter integrity is associated with gait disorders in cerebral small vessel disease. *Brain,* 134**,** 73-83.

de Leeuw, F. E., Richard, F., de Groot, J. C., van Duijn, C. M., Hofman, A., Van Gijn, J. & Breteler, M. M. 2004. Interaction between hypertension, apoE, and cerebral white matter lesions. *Stroke,* 35**,** 1057-60.

De Reuck, J., Auger, F., Cordonnier, C., Deramecourt, V., Durieux, N., Pasquier, F., Bordet, R., Maurage, C. A. & Leys, D. 2011. Comparison of 7.0-T T(2)*-magnetic resonance imaging of cerebral bleeds in post-mortem brain sections of Alzheimer patients with their neuropathological correlates. *Cerebrovasc Dis,* 31**,** 511-7.

Debette, S. & Markus, H. S. 2010. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ,* 341**,** c3666.

Delcourt, C., Huang, Y., Wang, J., Heeley, E., Lindley, R., Stapf, C., Tzourio, C., Arima, H., Parsons, M., Sun, J., Neal, B., Chalmers, J., Anderson, C. & Investigators, I. 2010. The second (main) phase of an open, randomised, multicentre study to investigate the effectiveness of an intensive blood pressure reduction in acute cerebral haemorrhage trial (INTERACT2). *Int J Stroke,* 5**,** 110-6.

Department of Health. 2013a. *Improving care for people with dementia* [Online]. London: https://www.gov.uk/government/policies/improving-care-for-people-with-dementia#actions.

Department of Health. 2013b. *G8 dementia summit – have your say on improving dementia research across the world* [Online]. http://dementiachallenge.dh.gov.uk/category/g8-dementia-summit/.

Derex, L., Nighoghossian, N., Hermier, M., Adeleine, P., Philippeau, F., Honnorat, J., Yilmaz, H., Dardel, P., Froment, J. C. & Trouillas, P. 2004. Thrombolysis for ischemic stroke in patients with old microbleeds on pretreatment MRI. *Cerebrovasc Dis,* 17**,** 238-41.

Dichgans, M., Mayer, M., Uttner, I., Bruning, R., Muller-Hocker, J., Rungger, G., Ebke, M., Klockgether, T. & Gasser, T. 1998. The phenotypic spectrum of CADASIL: clinical findings in 102 cases. *Ann Neurol,* 44**,** 731-9.

Dichgans, M., Holtmannspotter, M., Herzog, J., Peters, N., Bergmann, M. & Yousry, T. A. 2002. Cerebral microbleeds in CADASIL: a gradient-echo magnetic resonance imaging and autopsy study. *Stroke,* 33**,** 67-71.

Diener, H. C., Bogousslavsky, J., Brass, L. M., Cimminiello, C., Csiba, L., Kaste, M., Leys, D., Matias-Guiu, J., Rupprecht, H. J. & investigators, M. 2004. Aspirin and clopidogrel compared with clopidogrel alone after recent ischaemic stroke or transient ischaemic attack in high-risk patients (MATCH): randomised, double-blind, placebo-controlled trial. *Lancet,* 364**,** 331-7.

Dierksen, G. A., Skehan, M. E., Khan, M. A., Jeng, J., Nandigam, R. N., Becker, J. A., Kumar, A., Neal, K. L., Betensky, R. A., Frosch, M. P., Rosand, J., Johnson, K. A., Viswanathan, A., Salat, D. H. & Greenberg, S. M. 2010. Spatial relation between microbleeds and amyloid deposits in amyloid angiopathy. *Ann Neurol,* 68**,** 545-8.

Divry, P. 1941. De l'amyloidose vasculaire cérébrale et méningée (méningiopathie amyloide) dans la démence sénile. *J Belge Neurol Psychiatr* 42**,** 141-158.

Dotti, C. G. & De Strooper, B. 2009. Alzheimer's dementia by circulation disorders: when trees hide the forest. *Nat Cell Biol,* 11**,** 114-6.

Doubal, F. N., MacLullich, A. M., Ferguson, K. J., Dennis, M. S. & Wardlaw, J. M. 2010. Enlarged perivascular spaces on MRI are a feature of cerebral small vessel disease. *Stroke,* 41**,** 450-4.

Douglas, M. A. & Haerer, A. F. 1982. Long-term prognosis of hypertensive intracerebral hemorrhage. *Stroke,* 13**,** 488-91.

Dubas, F., Gray, F., Roullet, E. & Escourolle, R. 1985. [Arteriopathic leukoencephalopathy (17 anatomo-clinical cases)]. *Rev Neurol (Paris),* 141**,** 93-108.

Ellis, A. G. 1909. The pathogenesis of spontaneous cerebral hemorrhage. *Proc Pathol Soc Phil,* 12**,** 197–235.

Eppinger, H. 1887. Pathogenesis, Histogenesis and etiology of the aneurysms, including the aneurysm equi verminosum: pathological-anatomical studies. *Arch Fur kiln Chirurg,* xxxv.

Erkinjuntti, T., Ketonen, L., Sulkava, R., Sipponen, J., Vuorialho, M. & Iivanainen, M. 1987. Do white matter changes on MRI and CT differentiate vascular dementia from Alzheimer's disease? *J Neurol Neurosurg Psychiatry,* 50**,** 37-42.

Esiri, M. M. & Wilcock, G. K. 1986. Cerebral amyloid angiopathy in dementia and old age. *J Neurol Neurosurg Psychiatry,* 49**,** 1221-6.

Esiri, M. M., Wilcock, G. K. & Morris, J. H. 1997. Neuropathological assessment of the lesions of significance in vascular dementia. *J Neurol Neurosurg Psychiatry,* 63**,** 749-53.

Esiri, M. M., Nagy, Z., Smith, M. Z., Barnetson, L. & Smith, A. D. 1999. Cerebrovascular disease and threshold for dementia in the early stages of Alzheimer's disease. *Lancet,* 354**,** 919-20.

Etiene, D., Kraft, J., Ganju, N., Gomez-Isla, T., Gemelli, B., Hyman, B. T., Hedley-Whyte, E. T., Wands, J. R. & De La Monte, S. M. 1998. Cerebrovascular Pathology Contributes to the Heterogeneity of Alzheimer's Disease. *J Alzheimers Dis,* 1**,** 119-134.

Fan, Y. H., Zhang, L., Lam, W. W., Mok, V. C. & Wong, K. S. 2003. Cerebral microbleeds as a risk factor for subsequent intracerebral hemorrhages among patients with acute ischemic stroke. *Stroke,* 34**,** 2459-62.

Faulkner, W. 1996. *Basic principles of MRI*.

Fazekas, F., Chawluk, J. B., Alavi, A., Hurtig, H. I. & Zimmerman, R. A. 1987. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am J Roentgenol,* 149**,** 351-6.

Fazekas, F., Kleinert, R., Offenbacher, H., Payer, F., Schmidt, R., Kleinert, G., Radner, H. & Lechner, H. 1991. The morphologic correlate of incidental punctate white matter hyperintensities on MR images. *AJNR Am J Neuroradiol,* 12**,** 915-21.

Fazekas, F., Kleinert, R., Offenbacher, H., Schmidt, R., Kleinert, G., Payer, F., Radner, H. & Lechner, H. 1993. Pathologic correlates of incidental MRI white matter signal hyperintensities. *Neurology,* 43**,** 1683-9.

Fazekas, F., Kleinert, R., Roob, G., Kleinert, G., Kapeller, P., Schmidt, R. & Hartung, H. P. 1999. Histopathologic analysis of foci of signal loss on gradient-echo T2*-weighted MR images in patients with spontaneous intracerebral hemorrhage: evidence of microangiopathy-related microbleeds. *AJNR Am J Neuroradiol,* 20**,** 637-42.

Fernando, M.S., O'Brien, J.T., Perry, R.H., English, P., Forster, G., McMeekin, W., Slade, J.Y., Golkhar, A., Matthews, F.E., Barber, R., Kalaria, R.N., Ince, P.G.; Neuropathology Group of MRC CFAS. 2004. Comparison of the pathology of cerebral white matter with post-mortem magnetic resonance imaging (MRI) in the elderly brain. *Neuropathol Appl Neurobiol*, 30, 385-95.

Ferrarese, C. & Piazza, F. 2012. Vascular damage and neurodegeneration. *Journal of Alzheimer's Disease,* 29**,** 23-24.

Ferreiro, J. A., Ansbacher, L. E. & Vinters, H. V. 1989. Stroke related to cerebral amyloid angiopathy: the significance of systemic vascular disease. *J Neurol,* 236**,** 267-72.

Ferrer, I., Kaste, M. & Kalil, J. 2008. Vascular diseases. *In:* Love S, L. D., Ellison D, (ed.) *Greenfield's Neuropathology.* London: Arnold.

Ferrer, I. 2010. Cognitive impairment of vascular origin: neuropathology of cognitive impairment of vascular origin. *J Neurol Sci,* 299**,** 139-49.

Fiehler, J. 2006. Cerebral microbleeds: old leaks and new haemorrhages. *Int J Stroke,* 1**,** 122-30.

Fiehler, J., Albers, G. W., Boulanger, J. M., Derex, L., Gass, A., Hjort, N., Kim, J. S., Liebeskind, D. S., Neumann-Haefelin, T., Pedraza, S., Rother, J., Rothwell, P., Rovira, A., Schellinger, P. D. & Trenkler, J. 2007. Bleeding risk analysis in stroke imaging before thromboLysis (BRASIL): pooled analysis of T2*-weighted magnetic resonance imaging data from 570 patients. *Stroke,* 38**,** 2738-44.

Fischer, A. W. & Holfelder, H. 1930. lokales amyloid in Gehirn. *Dtsch Z Chir,* 227**,** 475-483.

Fisher, C. M. 1965. Lacunes: Small, Deep Cerebral Infarcts. *Neurology,* 15**,** 774-84.

Fisher, C. M. 1971a. Pathological observations in hypertensive cerebral hemorrhage. *J Neuropathol Exp Neurol,* 30**,** 536-50.

Fisher, C. M. 1972. Cerebral miliary aneurysms in hypertension. *Am J Pathol,* 66**,** 313-30.

Fisher, C. M. 1979. Capsular infarcts: the underlying vascular lesions. *Arch Neurol,* 36**,** 65-73.

Fisher, C. M. 2003. Hypertensive cerebral hemorrhage. Demonstration of the source of bleeding. *J Neuropathol Exp Neurol,* 62**,** 104-7.

Fisher, M. 1971b. The effect of corticosteroids on serum fungistatic activity. *Mycopathol Mycol Appl,* 44**,** 241-7.

Fisher, M., French, S., Ji, P. & Kim, R. C. 2010. Cerebral microbleeds in the elderly: a pathological analysis. *Stroke,* 41**,** 2782-5.

Fisher, M. 2010. The challenge of mixed cerebrovascular disease. *Ann N Y Acad Sci,* 1207**,** 18-22.

Fisher, M. 2012. Cerebral microbleeds and white matter disease: separated at birth? *Eur J Neurol,* 19**,** 2-3.

Flaherty, M. L., Kissela, B., Woo, D., Kleindorfer, D., Alwell, K., Sekar, P., Moomaw, C. J., Haverbusch, M. & Broderick, J. P. 2007. The increasing incidence of anticoagulant-associated intracerebral hemorrhage. *Neurology,* 68**,** 116-21.

Foerch, C., Sitzer, M., Steinmetz, H. & Neumann-Haefelin, T. 2006. Pretreatment with antiplatelet agents is not independently associated with unfavorable outcome in intracerebral hemorrhage. *Stroke,* 37**,** 2165-7.

Furuta, A., Ishii, N., Nishihara, Y. & Horie, A. 1991. Medullary arteries in aging and dementia. *Stroke,* 22**,** 442-6.

Gao, F.Q., Swartz, R.H., Scheltens, P., Leibovitch, F.S., Kiss, A., Honjo, K., Black, S.E. 2011. Complexity of MRI white matter hyperintensity assessments in relation to cognition in aging and dementia from the Sunnybrook Dementia Dtudy. J Alzheimers Dis, 26, 379-88.

Ge, L., Niu, G., Han, X., Gao, Y., Wu, Q., Wu, H., Zhang, Y. & Guo, D. 2011. Aspirin treatment increases the risk of cerebral microbleeds. *Can J Neurol Sci,* 38**,** 863-8.

Goldberg, S. 1950. Primary amyloidosis; report of a case and a review of the literature. *S Afr Med J,* 24**,** 801-4.

Goltz, R. W. 1952. Systematized amyloidosis. *Medicine (Baltimore),* 31**,** 381-409.

Good, C. D., Ng, V. W., Clifton, A., Britton, J. A., Hart, Y. & Wilkins, P. 1998. Amyloid angiopathy causing widespread miliary haemorrhages within the brain evident on MRI. *Neuroradiology,* 40**,** 308-11.

Goos, J. D., Kester, M. I., Barkhof, F., Klein, M., Blankenstein, M. A., Scheltens, P. & van der Flier, W. M. 2009. Patients with Alzheimer disease with multiple microbleeds: relation with cerebrospinal fluid biomarkers and cognition. *Stroke,* 40**,** 3455-60.

Goos, J. D., Henneman, W. J., Sluimer, J. D., Vrenken, H., Sluimer, I. C., Barkhof, F., Blankenstein, M. A., Scheltens, P. H. & van der Flier, W. M. 2010. Incidence of cerebral microbleeds: a longitudinal study in a memory clinic population. *Neurology,* 74**,** 1954-60.

Goos, J. D., van der Flier, W. M., Knol, D. L., Pouwels, P. J., Scheltens, P., Barkhof, F. & Wattjes, M. P. 2011. Clinical relevance of improved microbleed detection by susceptibility-weighted magnetic resonance imaging. *Stroke,* 42**,** 1894-900.

Gorelick, P. B. & Weisman, S. M. 2005. Risk of hemorrhagic stroke with aspirin use: an update. *Stroke,* 36**,** 1801-7.

Gorner, A., Lemmens, R., Schrooten, M. & Thijs, V. 2007. is leukoaraiosis on CT an accurate surrogate marker for the presence of microbleeds in acute stroke patients? *J Neurol,* 254**,** 284-9.

Gorter, J. W. 1999. Major bleeding during anticoagulation after cerebral ischemia: patterns and risk factors. Stroke Prevention In Reversible Ischemia Trial (SPIRIT). European Atrial Fibrillation Trial (EAFT) study groups. *Neurology,* 53**,** 1319-27.

Gould, D. B., Phalan, F. C., van Mil, S. E., Sundberg, J. P., Vahedi, K., Massin, P., Bousser, M. G., Heutink, P., Miner, J. H., Tournier-Lasserve, E. & John, S. W. 2006. Role of COL4A1 in small-vessel disease and hemorrhagic stroke. *N Engl J Med,* 354**,** 1489-96.

Gouw, A. A., Seewann, A., van der Flier, W. M., Barkhof, F., Rozemuller, A. M., Scheltens, P. & Geurts, J. J. 2011. Heterogeneity of small vessel disease: a systematic review of MRI and histopathology correlations. *J Neurol Neurosurg Psychiatry,* 82**,** 126-35.

Gravina, S. A., Ho, L., Eckman, C. B., Long, K. E., Otvos, L., Jr., Younkin, L. H., Suzuki, N. & Younkin, S. G. 1995. Amyloid beta protein (A beta) in Alzheimer's disease brain. Biochemical and immunocytochemical analysis with antibodies specific for forms ending at A beta 40 or A beta 42(43). *J Biol Chem,* 270**,** 7013-6.

Gray, F., Dubas, F., Roullet, E. & Escourolle, R. 1985. Leukoencephalopathy in diffuse hemorrhagic cerebral amyloid angiopathy. *Ann Neurol,* 18**,** 54-9.

Green, F. H. K. 1930. Miliary aneurysms in the brain. *J Pathol Bacteriol* 33**,** 71-7.

Greenberg, S. M., Vonsattel, J. P., Stakes, J. W., Gruber, M. & Finklestein, S. P. 1993. The clinical spectrum of cerebral amyloid angiopathy: presentations without lobar hemorrhage. *Neurology,* 43**,** 2073-9.

Greenberg, S. M., Finklestein, S. P. & Schaefer, P. W. 1996. Petechial hemorrhages accompanying lobar hemorrhage: detection by gradient-echo MRI. *Neurology,* 46**,** 1751-4.

Greenberg, S. M. 1998. Cerebral amyloid angiopathy: prospects for clinical diagnosis and treatment. *Neurology,* 51**,** 690-4.

Greenberg, S. M., Vonsattel, J. P., Segal, A. Z., Chiu, R. I., Clatworthy, A. E., Liao, A., Hyman, B. T. & Rebeck, G. W. 1998. Association of apolipoprotein E epsilon2 and vasculopathy in cerebral amyloid angiopathy. *Neurology,* 50**,** 961-5.

Greenberg, S. M., O'Donnell, H. C., Schaefer, P. W. & Kraft, E. 1999. MRI detection of new hemorrhages: potential marker of progression in cerebral amyloid angiopathy. *Neurology,* 53**,** 1135-8.

Greenberg, S. M., Eng, J. A., Ning, M., Smith, E. E. & Rosand, J. 2004a. Hemorrhage burden predicts recurrent intracerebral hemorrhage after lobar hemorrhage. *Stroke,* 35**,** 1415-20.

Greenberg, S. M., Gurol, M. E., Rosand, J. & Smith, E. E. 2004b. Amyloid angiopathy-related vascular cognitive impairment. *Stroke,* 35**,** 2616-9.

Greenberg, S. M. 2006. Small vessels, big problems. *N Engl J Med,* 354**,** 1451-3.

Greenberg, S. M., Nandigam, R. N., Delgado, P., Betensky, R. A., Rosand, J., Viswanathan, A., Frosch, M. P. & Smith, E. E. 2009a. Microbleeds versus macrobleeds: evidence for distinct entities. *Stroke,* 40**,** 2382-6.

Greenberg, S. M., Vernooij, M. W., Cordonnier, C., Viswanathan, A., Al-Shahi Salman, R., Warach, S., Launer, L. J., Van Buchem, M. A. & Breteler, M. M. 2009b. Cerebral microbleeds: a guide to detection and interpretation. *Lancet Neurol,* 8**,** 165-74.

Greene, G. M., Godersky, J. C., Biller, J., Hart, M. N. & Adams, H. P., Jr. 1990. Surgical experience with cerebral amyloid angiopathy. *Stroke,* 21**,** 1545-9.

Gregoire, S. M., Chaudhary, U. J., Brown, M. M., Yousry, T. A., Kallis, C., Jager, H. R. & Werring, D. J. 2009. The Microbleed Anatomical Rating Scale (MARS): reliability of a tool to map brain microbleeds. *Neurology,* 73**,** 1759-66.

Gregoire, S. M., Brown, M. M., Kallis, C., Jager, H. R., Yousry, T. A. & Werring, D. J. 2010a. MRI detection of new microbleeds in patients with ischemic stroke: five-year cohort follow-up study. *Stroke,* 41**,** 184-6.

Gregoire, S. M., Werring, D. J., Chaudhary, U. J., Thornton, J. S., Brown, M. M., Yousry, T. A. & Jager, H. R. 2010b. Choice of echo time on GRE T2*-weighted MRI influences the classification of brain microbleeds. *Clin Radiol,* 65**,** 391-4.

Gregoire, S. M., Jager, H. R., Yousry, T. A., Kallis, C., Brown, M. M. & Werring, D. J. 2010c. Brain microbleeds as a potential risk factor for antiplatelet-related intracerebral haemorrhage: hospital-based, case-control study. *J Neurol Neurosurg Psychiatry,* 81**,** 679-84.

Gregoire, S. M., Charidimou, A., Gadapa, N., Dolan, E., Antoun, N., Peeters, A., Vandermeeren, Y., Laloux, P., Baron, J. C., Jager, H. R. & Werring, D. J. 2011. Acute ischaemic brain lesions in intracerebral haemorrhage: multicentre cross-sectional magnetic resonance imaging study. *Brain,* 134**,** 2376-86.

Gregoire, S. M. & Werring, D. J. 2011. Defining and mapping cerebral microbleeds. *In:* Werring, D. J. (ed.) *Cerebral microbleeds: Pathophysiological to clinical practice.* New York: Cambridge University Press.

Gregoire, S. M., Smith, K., Jager, H. R., Benjamin, M., Kallis, C., Brown, M. M., Cipolotti, L. & Werring, D. J. 2012. Cerebral microbleeds and long-term cognitive outcome: longitudinal cohort study of stroke clinic patients. *Cerebrovasc Dis,* 33**,** 430-5.

Gregoire, S. M., Scheffler, G., Jager, H. R., Yousry, T. A., Brown, M. M., Kallis, C., Cipolotti, L. & Werring, D. J. 2013. Strictly lobar microbleeds are associated with executive impairment in patients with ischemic stroke or transient ischemic attack. *Stroke,* 44**,** 1267-72.

Gudmundsson, G., Hallgrimsson, J., Jonasson, T. A. & Bjarnason, O. 1972. Hereditary cerebral haemorrhage with amyloidosis. *Brain,* 95**,** 387-404.

Guo, D. C., Papke, C. L., Tran-Fadulu, V., Regalado, E. S., Avidan, N., Johnson, R. J., Kim, D. H., Pannu, H., Willing, M. C., Sparks, E., Pyeritz, R. E., Singh, M. N., Dalman, R. L., Grotta, J. C., Marian, A. J., Boerwinkle, E. A., Frazier, L. Q., LeMaire, S. A., Coselli, J. S., Estrera, A. L., Safi, H. J., Veeraraghavan, S., Muzny, D. M., Wheeler, D. A., Willerson, J. T., Yu, R. K., Shete, S. S., Scherer, S. E., Raman, C. S., Buja, L. M. & Milewicz, D. M. 2009. Mutations in smooth muscle alpha-actin (ACTA2) cause coronary artery disease, stroke, and Moyamoya disease, along with thoracic aortic disease. *Am J Hum Genet,* 84**,** 617-27.

Gustafsson, C., Asplund, K., Britton, M., Norrving, B., Olsson, B. & Marke, L. A. 1992. Cost effectiveness of primary stroke prevention in atrial fibrillation: Swedish national perspective. *BMJ,* 305**,** 1457-60.

Haacke, E. M., Xu, Y., Cheng, Y. C. & Reichenbach, J. R. 2004. Susceptibility weighted imaging (SWI). *Magn Reson Med,* 52**,** 612-8.

Haacke, E. M., DelProposto, Z. S., Chaturvedi, S., Sehgal, V., Tenzer, M., Neelavalli, J. & Kido, D. 2007. Imaging cerebral amyloid angiopathy with susceptibility-weighted imaging. *AJNR Am J Neuroradiol,* 28**,** 316-7.

Haacke, E. M., Boikov, A. S., Barnes, S., Neejavalli, J. & Khan, M. A. 2011. Susceptibility – weighted imaging. *In:* Werring, D. J. (ed.) *Cerebral microbleeds: Pathophysiological to clinical practice.* Cambridge University Press.

Hachinski, V., Iadecola, C., Petersen, R. C., Breteler, M. M., Nyenhuis, D. L., Black, S. E., Powers, W. J., DeCarli, C., Merino, J. G., Kalaria, R. N., Vinters, H. V., Holtzman, D. M., Rosenberg, G. A., Wallin, A., Dichgans, M., Marler, J. R. & Leblanc, G. G. 2006. National Institute of Neurological Disorders and Stroke-Canadian Stroke Network vascular cognitive impairment harmonization standards. *Stroke,* 37**,** 2220-41.

Hachinski, V. 2007. The 2005 Thomas Willis Lecture: stroke and vascular cognitive impairment: a transdisciplinary, translational and transactional approach. *Stroke,* 38**,** 1396-1403.

Hachinski, V. 2011. Stroke and Alzheimer disease: fellow travelers or partners in crime? *Arch Neurol,* 68**,** 797-8.

Hachinski, V. C., Potter, P. & Merskey, H. 1987. Leuko-araiosis. *Arch Neurol,* 44**,** 21-3.

Haglund, M. & Englund, E. 2002. Cerebral amyloid angiopathy, white matter lesions and Alzheimer encephalopathy - a histopathological assessment. *Dement Geriatr Cogn Disord,* 14**,** 161-6.

Haglund, M., Kalaria, R., Slade, J. Y. & Englund, E. 2006a. Differential deposition of amyloid beta peptides in cerebral amyloid angiopathy associated with Alzheimer's disease and vascular dementia. *Acta Neuropathol,* 111**,** 430-5.

Haglund, M., Passant, U., Sjobeck, M., Ghebremedhin, E. & Englund, E. 2006b. Cerebral amyloid angiopathy and cortical microinfarcts as putative substrates of vascular dementia. *Int J Geriatr Psychiatry,* 21**,** 681-7.

Haley, A. P., Hoth, K. F., Gunstad, J., Paul, R. H., Jefferson, A. L., Tate, D. F., Ono, M., Jerskey, B. A., Poppas, A., Sweet, L. H. & Cohen, R. A. 2009. Subjective cognitive complaints relate to white matter hyperintensities and future cognitive decline in patients with cardiovascular disease. *Am J Geriatr Psychiatry,* 17**,** 976-85.

Han, J., Gao, P., Lin, Y., Zhang, J., Xu, L. & Xue, J. 2009. Three-tesla magnetic resonance imaging study of cerebral microbleeds in patients with ischemic stroke. *Neurol Res,* 31**,** 900-3.

Hanyu, H., Tanaka, Y., Shimizu, S., Takasaki, M. & Abe, K. 2003a. Cerebral microbleeds in Alzheimer's disease. *J Neurol,* 250**,** 1496-7.

Hanyu, H., Tanaka, Y., Shimizu, S., Takasaki, M., Fujita, H., Kaneko, N., Yamamoto, Y. & Harada, M. 2003b. Cerebral microbleeds in Binswanger's disease: a gradient-echo T2*-weighted magnetic resonance imaging study. *Neurosci Lett,* 340**,** 213-6.

Harb, R., Whiteus, C., Freitas, C. & Grutzendler, J. 2013. In vivo imaging of cerebral microvascular plasticity from birth to death. *J Cereb Blood Flow Metab,* 33**,** 146-56.

Hart, R. G., Boop, B. S. & Anderson, D. C. 1995. Oral anticoagulants and intracranial hemorrhage. Facts and hypotheses. *Stroke,* 26**,** 1471-7.

Hart, R. G., Tonarelli, S. B. & Pearce, L. A. 2005. Avoiding central nervous system bleeding during antithrombotic therapy: recent data and ideas. *Stroke,* 36**,** 1588-93.

Hassan, A., Lansbury, A., Catto, A. J., Guthrie, A., Spencer, J., Craven, C., Grant, P. J. & Bamford, J. M. 2002. Angiotensin converting enzyme insertion/deletion genotype is associated with leukoaraiosis in lacunar syndromes. *J Neurol Neurosurg Psychiatry,* 72**,** 343-6.

He, J., Whelton, P. K., Vu, B. & Klag, M. J. 1998. Aspirin and risk of hemorrhagic stroke: a meta-analysis of randomized controlled trials. *JAMA,* 280**,** 1930-5.

Henneman, W. J., Sluimer, J. D., Cordonnier, C., Baak, M. M., Scheltens, P., Barkhof, F. & van der Flier, W. M. 2009. MRI biomarkers of vascular damage and atrophy predicting mortality in a memory clinic population. *Stroke,* 40**,** 492-8.

Hennerici, M. G. 2009. What are the mechanisms for post-stroke dementia? *Lancet Neurol,* 8**,** 973-5.

Henon, H., Pasquier, F., Durieu, I., Pruvo, J. P. & Leys, D. 1998. Medial temporal lobe atrophy in stroke patients: relation to pre-existing dementia. *J Neurol Neurosurg Psychiatry,* 65**,** 641-7.

Henon, H., Pasquier, F. & Leys, D. 2006. Poststroke dementia. *Cerebrovasc Dis,* 22**,** 61-70.

Henskens, L. H., van Oostenbrugge, R. J., Kroon, A. A., de Leeuw, P. W. & Lodder, J. 2008. Brain microbleeds are associated with ambulatory blood pressure levels in a hypertensive population. *Hypertension,* 51**,** 62-8.

Henskens, L. H., van Oostenbrugge, R. J., Kroon, A. A., Hofman, P. A., Lodder, J. & de Leeuw, P. W. 2009. Detection of silent cerebrovascular disease refines risk stratification of hypertensive patients. *J Hypertens,* 27**,** 846-53.

Higashi, M. K., Veenstra, D. L., Kondo, L. M., Wittkowsky, A. K., Srinouanprachanh, S. L., Farin, F. M. & Rettie, A. E. 2002. Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA,* 287**,** 1690-8.

Higgins, W. H. & Higgins, W. H., Jr. 1950. Primary amyloidosis; a clinical and pathological study. *Am J Med Sci,* 220**,** 610-5.

Hinton, D. R., Dolan, E. & Sima, A. A. 1984. The value of histopathological examination of surgically removed blood clot in determining the etiology of spontaneous intracerebral hemorrhage. *Stroke,* 15**,** 517-20.

Hirabuki, N., Fujita, N., Fujii, K., Hashimoto, T. & Kozuka, T. 1994. MR appearance of Virchow-Robin spaces along lenticulostriate arteries: spin-echo and two-dimensional fast low-angle shot imaging. *AJNR Am J Neuroradiol,* 15**,** 277-81.

Hirono, N., Kitagaki, H., Kazui, H., Hashimoto, M. & Mori, E. 2000. Impact of white matter changes on clinical manifestation of Alzheimer's disease: A quantitative study. *Stroke,* 31**,** 2182-8.

Holtzman, D. M., Bales, K. R., Tenkova, T., Fagan, A. M., Parsadanian, M., Sartorius, L. J., Mackey, B., Olney, J., McKeel, D., Wozniak, D. & Paul, S. M. 2000. Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A,* 97**,** 2892-7.

Hortobagyi, T. & Al-Sarraj, S. 2008. The Significance of Diffuse Axonal Injury: how to diagnose it and what does it tell us? *Advances in Clinical Neuroscience and Rehabilitation,* 8**,** 16-18.

Huang, Y., Cheng, Y., Wu, J., Li, Y., Xu, E., Hong, Z., Li, Z., Zhang, W., Ding, M., Gao, X., Fan, D., Zeng, J., Wong, K., Lu, C., Xiao, J., Yao, C. & Cilostazol versus Aspirin for Secondary Ischaemic Stroke Prevention cooperation, i. 2008. Cilostazol as an alternative to aspirin after ischaemic stroke: a randomised, double-blind, pilot study. *Lancet Neurol,* 7**,** 494-9.

Iadecola, C. 2004. Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat Rev Neurosci,* 5**,** 347-60.

Iadecola, C., Park, L. & Capone, C. 2009. Threats to the mind: aging, amyloid, and hypertension. *Stroke,* 40**,** S40-4.

Igase, M., Tabara, Y., Igase, K., Nagai, T., Ochi, N., Kido, T., Nakura, J., Sadamoto, K., Kohara, K. & Miki, T. 2009. Asymptomatic cerebral microbleeds seen in healthy subjects have a strong association with asymptomatic lacunar infarction. *Circ J,* 73**,** 530-3.

Imaizumi, T., Chiba, M., Honma, T., Yoshikawa, J. & Niwa, J. 2003. Dynamics of dotlike hemosiderin spots associated with intracerebral hemorrhage. *J Neuroimaging,* 13**,** 155-7.

Imaizumi, T., Horita, Y., Hashimoto, Y. & Niwa, J. 2004. Dotlike hemosiderin spots on T2*-weighted magnetic resonance imaging as a predictor of stroke recurrence: a prospective study. *J Neurosurg,* 101**,** 915-20.

Imaizumi, T., Honma, T., Horita, Y., Kawamura, M., Kohama, I., Miyata, K., Nyon, K. S. & Niwa, J. 2008. The number of microbleeds on gradient T2*-weighted magnetic resonance image at the onset of intracerebral hemorrhage. *J Stroke Cerebrovasc Dis,* 17**,** 30-4.

Inzitari, D., Pracucci, G., Poggesi, A., Carlucci, G., Barkhof, F., Chabriat, H., Erkinjuntti, T., Fazekas, F., Ferro, J. M., Hennerici, M., Langhorne, P., O'Brien, J., Scheltens, P., Visser, M. C., Wahlund, L. O., Waldemar, G., Wallin, A., Pantoni, L. & Group, L. S. 2009. Changes in white matter as determinant of global functional decline in older independent outpatients: three year follow-up of LADIS (leukoaraiosis and disability) study cohort. *BMJ,* 339**,** b2477.

Ishii, N., Nishihara, Y. & Horie, A. 1984. Amyloid angiopathy and lobar cerebral haemorrhage. *J Neurol Neurosurg Psychiatry,* 47**,** 1203-10.

Ishikawa, T., Kuroda, S., Nakayama, N., Terae, S., Kudou, K. & Iwasaki, Y. 2005. Prevalence of asymptomatic microbleeds in patients with moyamoya disease. *Neurol Med Chir (Tokyo),* 45**,** 495-500; discussion 500.

Izenberg, A., Aviv, R. I., Demaerschalk, B. M., Dodick, D. W., Hopyan, J., Black, S. E. & Gladstone, D. J. 2009. Crescendo transient Aura attacks: a transient ischemic attack mimic caused by focal subarachnoid hemorrhage. *Stroke,* 40**,** 3725-9.

Jackson, E. F., Ginsberg, L. E., Schomer, D. F. & Leeds, N. E. 1997. A review of MRI pulse sequences and techniques in neuroimaging. *Surg Neurol,* 47**,** 185-99.

James, T. H., Ramadan, H., Gregoire, S. M., Mufti, S., Lipman, G., Jäger, H. R., Losseff, N. A. & Werring, D. J. 2011. Can cerebral microbleeds cause an acute stroke syndrome? . *Neurol Clin Pract,* 1**,** 75-77.

Janaway, B. M., Simpson, J. E., Hoggard, N., Highley, J. R., Forster, G., Drew, D., Gebril, O. H., Matthews, F. E., Brayne, C., Wharton, S. B., Ince, P. G., on behalf of the, M. R. C. C. F. & Ageing Neuropathology, S. 2013. Brain haemosiderin in older people: pathological evidence for an ischaemic origin of MRI microbleeds. *Neuropathol Appl Neurobiol*.

Jannes, J., Hamilton-Bruce, M. A., Pilotto, L., Smith, B. J., Mullighan, C. G., Bardy, P. G. & Koblar, S. A. 2004. Tissue plasminogen activator -7351C/T enhancer polymorphism is a risk factor for lacunar stroke. *Stroke,* 35**,** 1090-4.

Jeerakathil, T., Wolf, P. A., Beiser, A., Hald, J. K., Au, R., Kase, C. S., Massaro, J. M. & DeCarli, C. 2004a. Cerebral microbleeds: prevalence and associations with cardiovascular risk factors in the Framingham Study. *Stroke,* 35**,** 1831-5.

Jeerakathil, T., Wolf, P. A., Beiser, A., Massaro, J., Seshadri, S., D'Agostino, R. B. & DeCarli, C. 2004b. Stroke risk profile predicts white matter hyperintensity volume: the Framingham Study. *Stroke,* 35**,** 1857-61.

Jellinger, K. 1977. Cerebrovascular amyloidosis with cerebral hemorrhage. *J Neurol,* 214**,** 195-206.

Jellinger, K. A. 2002. Alzheimer disease and cerebrovascular pathology: an update. *J Neural Transm,* 109**,** 813-36.

Jellinger, K. A. & Mitter-Ferstl, E. 2003. The impact of cerebrovascular lesions in Alzheimer disease--a comparative autopsy study. *J Neurol,* 250**,** 1050-5.

Jellinger, K. A. 2007. The enigma of vascular cognitive disorder and vascular dementia. *Acta Neuropathol,* 113**,** 349-88.

Jellinger, K. A. 2013a. Pathology and pathogenesis of vascular cognitive impairment-a critical update. *Front Aging Neurosci,* 5**,** 17.

Jellinger, K. A. 2013b. Challenges in the Neuropathological Diagnosis of Dementias. *International Journal of Neuropathology,* 1**,** 8-52

Jeon, S. B. & Kang, D. W. 2007. Neurological picture. Cerebral air emboli on T2-weighted gradient-echo magnetic resonance imaging. *J Neurol Neurosurg Psychiatry,* 78**,** 871.

Jeon, S. B., Kang, D. W., Cho, A. H., Lee, E. M., Choi, C. G., Kwon, S. U. & Kim, J. S. 2007. Initial microbleeds at MR imaging can predict recurrent intracerebral hemorrhage. *J Neurol,* 254**,** 508-12.

Jeon, S. B., Kwon, S. U., Cho, A. H., Yun, S. C., Kim, J. S. & Kang, D. W. 2009. Rapid appearance of new cerebral microbleeds after acute ischemic stroke. *Neurology,* 73**,** 1638-44.

Jeon, S. B., Lee, J. W., Kim, S. J., Chung, C. H., Kwon, S. U., Choi, C. G., Choo, S. J., Nah, H. W., Kim, J. S. & Kang, D. W. 2010. New cerebral lesions on T2*-weighted gradient-echo imaging after cardiac valve surgery. *Cerebrovasc Dis,* 30**,** 194-9.

Jeong, S. W., Jung, K. H., Chu, K., Bae, H. J., Lee, S. H. & Roh, J. K. 2004. Clinical and radiologic differences between primary intracerebral hemorrhage with and without microbleeds on gradient-echo magnetic resonance images. *Arch Neurol,* 61**,** 905-9.

Johnson, K. A., Gregas, M., Becker, J. A., Kinnecom, C., Salat, D. H., Moran, E. K., Smith, E. E., Rosand, J., Rentz, D. M., Klunk, W. E., Mathis, C. A., Price, J. C., Dekosky, S. T.,

Fischman, A. J. & Greenberg, S. M. 2007. Imaging of amyloid burden and distribution in cerebral amyloid angiopathy. *Ann Neurol,* 62**,** 229-34.

Jokinen, H., Kalska, H., Mantyla, R., Ylikoski, R., Hietanen, M., Pohjasvaara, T., Kaste, M. & Erkinjuntti, T. 2005. White matter hyperintensities as a predictor of neuropsychological deficits post-stroke. *J Neurol Neurosurg Psychiatry,* 76**,** 1229-33.

Jokinen, H., Kalska, H., Ylikoski, R., Madureira, S., Verdelho, A., van der Flier, W. M., Scheltens, P., Barkhof, F., Visser, M. C., Fazekas, F., Schmidt, R., O'Brien, J., Waldemar, G., Wallin, A., Chabriat, H., Pantoni, L., Inzitari, D. & Erkinjuntti, T. 2009. Longitudinal cognitive decline in subcortical ischemic vascular disease--the LADIS Study. *Cerebrovasc Dis,* 27**,** 384-91.

Jones, K. M., Mulkern, R. V., Schwartz, R. B., Oshio, K., Barnes, P. D. & Jolesz, F. A. 1992. Fast spin-echo MR imaging of the brain and spine: current concepts. *AJR Am J Roentgenol,* 158**,** 1313-20.

Josephson, C.B., Frantazias, J., Samarasekera, N., Al-Shahi Salman, E. 2010. The persisting burden of intracerebral hemorrhage: can effective treatments be found? *PLoS Med*, 10, 1-6.

Joutel, A., Corpechot, C., Ducros, A., Vahedi, K., Chabriat, H., Mouton, P., Alamowitch, S., Domenga, V., Cecillion, M., Marechal, E., Maciazek, J., Vayssiere, C., Cruaud, C., Cabanis, E. A., Ruchoux, M. M., Weissenbach, J., Bach, J. F., Bousser, M. G. & Tournier-Lasserve, E. 1996. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature,* 383**,** 707-10.

Jouvent, E., Poupon, C., Gray, F., Paquet, C., Mangin, J. F., Le Bihan, D. & Chabriat, H. 2011. Intracortical infarcts in small vessel disease: a combined 7-T postmortem MRI and neuropathological case study in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Stroke,* 42**,** e27-30.

Julien, J., Vital, C., Lagueny, A., Ferrer, X. & Vital, A. 1983. [Recurrent cerebral hemorrhage and amyloid angiopathy]. *Rev Neurol (Paris),* 139**,** 763-7.

Kakuda, W., Thijs, V. N., Lansberg, M. G., Bammer, R., Wechsler, L., Kemp, S., Moseley, M. E., Marks, M. P. & Albers, G. W. 2005. Clinical importance of microbleeds in patients receiving IV thrombolysis. *Neurology,* 65**,** 1175-8.

Kang, D. W., Latour, L. L., Chalela, J. A., Dambrosia, J. A. & Warach, S. 2004. Early and late recurrence of ischemic lesion on MRI: evidence for a prolonged stroke-prone state? *Neurology,* 63**,** 2261-5.

Kato, H., Izumiyama, M., Izumiyama, K., Takahashi, A. & Itoyama, Y. 2002. Silent cerebral microbleeds on T2*-weighted MRI: correlation with stroke subtype, stroke recurrence, and leukoaraiosis. *Stroke,* 33**,** 1536-40.

Keage, H. A., Carare, R. O., Friedland, R. P., Ince, P. G., Love, S., Nicoll, J. A., Wharton, S. B., Weller, R. O. & Brayne, C. 2009. Population studies of sporadic cerebral amyloid angiopathy and dementia: a systematic review. *BMC Neurol,* 9**,** 3.

Kemper, T. L. 1994. Neuroanatomical and neuropathological changes during aging and dementia. *In:* Albert, M. L. & Knoefel, J. E. (eds.) *Clinical Neurology of Aging.* 2nd ed. New York: Oxford University Press

Khan, M. A., Viswanathan, A. & Greenberg, S. M. 2011. Cerebral microbleeds in relation to cerebral amyloid angiopathy. *In:* Werring, D. J. (ed.) *Cerebral microbleeds: Pathophysiological to clinical practice.* New York: Cambridge University Press.

Kidwell, C. S., Lees, K. R., Muir, K. W., Chen, C., Davis, S. M., De Silva, D. A., Weir, C. J., Starkman, S., Alger, J. R., Saver, J. L. & Investigators, M. I. 2009. Results of the MRI substudy of the intravenous magnesium efficacy in stroke trial. *Stroke,* 40**,** 1704-9.

Kidwell, C. S. & Greenberg, S. M. 2009. Red meets white: do microbleeds link hemorrhagic and ischemic cerebrovascular disease? *Neurology,* 73**,** 1614-5.

Kikuta, K., Takagi, Y., Nozaki, K., Hanakawa, T., Okada, T., Mikuni, N., Miki, Y., Fushmi, Y., Yamamoto, A., Yamada, K., Fukuyama, H. & Hashimoto, N. 2005. Asymptomatic microbleeds in moyamoya disease: T2*-weighted gradient-echo magnetic resonance imaging study. *J Neurosurg,* 102**,** 470-5.

Kikuta, K., Takagi, Y., Nozaki, K., Okada, T. & Hashimoto, N. 2007. Histological analysis of microbleed after surgical resection in a patient with moyamoya disease. *Neurol Med Chir (Tokyo),* 47**,** 564-7.

Kikuta, K., Takagi, Y., Nozaki, K., Sawamoto, N., Fukuyama, H. & Hashimoto, N. 2008. The presence of multiple microbleeds as a predictor of subsequent cerebral hemorrhage in patients with moyamoya disease. *Neurosurgery,* 62**,** 104-11, discussion 111-2.

Kilander, L., Nyman, H., Boberg, M., Hansson, L. & Lithell, H. 1998. Hypertension is related to cognitive impairment: a 20-year follow-up of 999 men. *Hypertension,* 31**,** 780-6.

Kim, C. K., Kwon, H. T. & Kwon, H. M. 2012. No significant association of aspirin use with cerebral microbleeds in the asymptomatic elderly. *J Neurol Sci,* 319**,** 56-8.

Kim, M., Bae, H. J., Lee, J., Kang, L., Lee, S., Kim, S., Lee, J. E., Lee, K. M., Yoon, B. W., Kwon, O., Koo, J. S. & Kim, B. K. 2005. APOE epsilon2/epsilon4 polymorphism and cerebral microbleeds on gradient-echo MRI. *Neurology,* 65**,** 1474-5.

Kimberly, W. T., Gilson, A., Rost, N. S., Rosand, J., Viswanathan, A., Smith, E. E. & Greenberg, S. M. 2009. Silent ischemic infarcts are associated with hemorrhage burden in cerebral amyloid angiopathy. *Neurology,* 72**,** 1230-5.

Kirsch, W., McAuley, G., Holshouser, B., Petersen, F., Ayaz, M., Vinters, H. V., Dickson, C., Haacke, E. M., Britt, W., 3rd, Larseng, J., Kim, I., Mueller, C., Schrag, M. & Kido, D. 2009. Serial susceptibility weighted MRI measures brain iron and microbleeds in dementia. *J Alzheimers Dis,* 17**,** 599-609.

Klein, I., Iung, B., Labreuche, J., Hess, A., Wolff, M., Messika-Zeitoun, D., Lavallee, P., Laissy, J. P., Leport, C., Duval, X. & Group, I. S. 2009. Cerebral microbleeds are frequent in infective endocarditis: a case-control study. *Stroke,* 40**,** 3461-5.

Klunk, W. E., Engler, H., Nordberg, A., Wang, Y., Blomqvist, G., Holt, D. P., Bergstrom, M., Savitcheva, I., Huang, G. F., Estrada, S., Ausen, B., Debnath, M. L., Barletta, J., Price, J. C., Sandell, J., Lopresti, B. J., Wall, A., Koivisto, P., Antoni, G., Mathis, C. A. & Langstrom, B. 2004. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann Neurol,* 55**,** 306-19.

Knopman, D., Boland, L. L., Mosley, T., Howard, G., Liao, D., Szklo, M., McGovern, P. & Folsom, A. R. 2001. Cardiovascular risk factors and cognitive decline in middle-aged adults. *Neurology,* 56**,** 42-8.

Knottnerus, I. L., Gielen, M., Lodder, J., Rouhl, R. P., Staals, J., Vlietinck, R. & van Oostenbrugge, R. J. 2011. Family history of stroke is an independent risk factor for lacunar stroke subtype with asymptomatic lacunar infarcts at younger ages. *Stroke,* 42**,** 1196-200.

Knudsen, K. A., Rosand, J., Karluk, D. & Greenberg, S. M. 2001. Clinical diagnosis of cerebral amyloid angiopathy: validation of the Boston criteria. *Neurology,* 56**,** 537-9.

Koennecke, H. C. 2006. Cerebral microbleeds on MRI: prevalence, associations, and potential clinical implications. *Neurology,* 66**,** 165-71.

Kramer, J. H., Reed, B. R., Mungas, D., Weiner, M. W. & Chui, H. C. 2002. Executive dysfunction in subcortical ischaemic vascular disease. *J Neurol Neurosurg Psychiatry,* 72**,** 217-20.

Kronmal, R. A., Hart, R. G., Manolio, T. A., Talbert, R. L., Beauchamp, N. J. & Newman, A. 1998. Aspirin use and incident stroke in the cardiovascular health study. CHS Collaborative Research Group. *Stroke,* 29**,** 887-94.

Kumar, S., Goddeau, R. P., Jr., Selim, M. H., Thomas, A., Schlaug, G., Alhazzani, A., Searls, D. E. & Caplan, L. R. 2010. Atraumatic convexal subarachnoid hemorrhage: clinical presentation, imaging patterns, and etiologies. *Neurology,* 74**,** 893-9.

Kwa, V. I., Franke, C. L., Verbeeten, B., Jr. & Stam, J. 1998. Silent intracerebral microhemorrhages in patients with ischemic stroke. Amsterdam Vascular Medicine Group. *Ann Neurol,* 44**,** 372-7.

Kwee, R. M. & Kwee, T. C. 2007. Virchow-Robin spaces at MR imaging. *Radiographics,* 27**,** 1071-86.

Kwon, H. M., Kim, B. J., Lee, S. H., Choi, S. H., Oh, B. H. & Yoon, B. W. 2006. Metabolic syndrome as an independent risk factor of silent brain infarction in healthy people. *Stroke,* 37**,** 466-70.

Lambert, M. P., Barlow, A. K., Chromy, B. A., Edwards, C., Freed, R., Liosatos, M., Morgan, T. E., Rozovsky, I., Trommer, B., Viola, K. L., Wals, P., Zhang, C., Finch, C. E., Krafft, G. A. & Klein, W. L. 1998. Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci U S A,* 95**,** 6448-53.

Lammie, G. A. 2000. Pathology of small vessel stroke. *Br Med Bull,* 56**,** 296-306.

Lammie, G. A. 2002. Hypertensive cerebral small vessel disease and stroke. *Brain Pathol,* 12**,** 358-70.

Landis, J. R. & Koch, G. G. 1977. The measurement of observer agreement for categorical data. *Biometrics,* 33**,** 159-74.

Launer, L. J. 2011. Risk factors for cerebral microbleeds. *In:* Werring, D. J. (ed.) *Cerebral microbleeds: Pathophysiological to clinical practice.* New York: Cambridge University Press.

Lauterbur, P. C. 1973. Image formation by induced local interactions. Examples employing nuclear magnetic resonance. 1973. *Nature***,** 190-191.

Lee, G. H., Kwon, S. U. & Kang, D. W. 2008a. Warfarin-induced intracerebral hemorrhage associated with microbleeds. *J Clin Neurol,* 4**,** 131-3.

Lee, S. H., Bae, H. J., Yoon, B. W., Kim, H., Kim, D. E. & Roh, J. K. 2002. Low concentration of serum total cholesterol is associated with multifocal signal loss lesions on gradient-echo magnetic resonance imaging: analysis of risk factors for multifocal signal loss lesions. *Stroke,* 33**,** 2845-9.

Lee, S. H., Kwon, S. J., Kim, K. S., Yoon, B. W. & Roh, J. K. 2004a. Topographical distribution of pontocerebellar microbleeds. *AJNR Am J Neuroradiol,* 25**,** 1337-41.

Lee, S. H., Park, J. M., Kwon, S. J., Kim, H., Kim, Y. H., Roh, J. K. & Yoon, B. W. 2004b. Left ventricular hypertrophy is associated with cerebral microbleeds in hypertensive patients. *Neurology,* 63**,** 16-21.

Lee, S. H., Bae, H. J., Kwon, S. J., Kim, H., Kim, Y. H., Yoon, B. W. & Roh, J. K. 2004c. Cerebral microbleeds are regionally associated with intracerebral hemorrhage. *Neurology,* 62**,** 72-6.

Lee, S. H., Heo, J. H. & Yoon, B. W. 2005. Effects of microbleeds on hemorrhage development in leukoaraiosis patients. *Hypertens Res,* 28**,** 895-9.

Lee, S. H., Kim, B. J. & Roh, J. K. 2006. Silent microbleeds are associated with volume of primary intracerebral hemorrhage. *Neurology,* 66**,** 430-2.

Lee, S. H., Kim, S. M., Kim, N., Yoon, B. W. & Roh, J. K. 2007. Cortico-subcortical distribution of microbleeds is different between hypertension and cerebral amyloid angiopathy. *J Neurol Sci,* 258**,** 111-4.

Lee, S. H., Kang, B. S., Kim, N. & Roh, J. K. 2008b. Does microbleed predict haemorrhagic transformation after acute atherothrombotic or cardioembolic stroke? *J Neurol Neurosurg Psychiatry,* 79**,** 913-6.

Lee, S. H., Ryu, W. S. & Roh, J. K. 2009. Cerebral microbleeds are a risk factor for warfarin-related intracerebral hemorrhage. *Neurology,* 72**,** 171-6.

Lee, S. H. & Roh, J. K. 2011. Cerebral microbleeds in relation to cerebrovascular disease. *In:* Werring, D. J. (ed.) *Cerebral microbleeds: Pathophysiological to clinical practice.* New York: Cambridge University Press.

Lee, S. S. & Stemmermann, G. N. 1978. Congophilic angiopathy and cerebral hemorrhage. *Arch Pathol Lab Med,* 102**,** 317-21.

Lemmens, R., Gorner, A., Schrooten, M. & Thijs, V. 2007. Association of apolipoprotein E epsilon2 with white matter disease but not with microbleeds. *Stroke,* 38**,** 1185-8.

Lesnik Oberstein, S. A., van den Boom, R., van Buchem, M. A., van Houwelingen, H. C., Bakker, E., Vollebregt, E., Ferrari, M. D., Breuning, M. H. & Haan, J. 2001. Cerebral microbleeds in CADASIL. *Neurology,* 57**,** 1066-70.

Leys, D., Henon, H., Mackowiak-Cordoliani, M. A. & Pasquier, F. 2005. Poststroke dementia. *Lancet Neurol,* 4**,** 752-9.

Leys, D. & Cordonnier, C. 2010. Brain microbleeds as a potential risk factor for antiplatelet-related intracerebral haemorrhage. *J Neurol Neurosurg Psychiatry,* 81**,** 589-90.

Leys, D., Soetaert, G., Petit, H., Fauquette, A. , Pruvo, J.P., Steinling, M. 1990. Periventricular and white matter magnetic resonance imaging hyperintensities do not differ between Alzheimer's disease and normal aging. *Arch Neurol*. 47:524-7.

Liem, M. K., Lesnik Oberstein, S. A., Haan, J., van der Neut, I. L., Ferrari, M. D., van Buchem, M. A., Middelkoop, H. A. & van der Grond, J. 2009. MRI correlates of cognitive decline in CADASIL: a 7-year follow-up study. *Neurology,* 72**,** 143-8.

Lim, J. B. & Kim, E. 2009. Silent microbleeds and old hematomas in spontaneous cerebral hemorrhages. *J Korean Neurosurg Soc,* 46**,** 38-44.

Linden, T., Skoog, I., Fagerberg, B., Steen, B. & Blomstrand, C. 2004. Cognitive impairment and dementia 20 months after stroke. *Neuroepidemiology,* 23**,** 45-52.

Linn, J., Herms, J., Dichgans, M., Bruckmann, H., Fesl, G., Freilinger, T. & Wiesmann, M. 2008. Subarachnoid hemosiderosis and superficial cortical hemosiderosis in cerebral amyloid angiopathy. *AJNR Am J Neuroradiol,* 29**,** 184-6.

Linn, J., Halpin, A., Demaerel, P., Ruhland, J., Giese, A. D., Dichgans, M., van Buchem, M. A., Bruckmann, H. & Greenberg, S. M. 2010. Prevalence of superficial siderosis in patients with cerebral amyloid angiopathy. *Neurology,* 74**,** 1346-50.

Lip, G. Y., Tse, H. F. & Lane, D. A. 2012. Atrial fibrillation. *Lancet,* 379**,** 648-61.

Longstreth, W. T., Jr., Bernick, C., Manolio, T. A., Bryan, N., Jungreis, C. A. & Price, T. R. 1998. Lacunar infarcts defined by magnetic resonance imaging of 3660 elderly people: the Cardiovascular Health Study. *Arch Neurol,* 55**,** 1217-25.

Lovelock, C. E., Molyneux, A. J. & Rothwell, P. M. 2007. Change in incidence and aetiology of intracerebral haemorrhage in Oxfordshire, UK, between 1981 and 2006: a population-based study. *Lancet Neurol,* 6**,** 487-93.

Lovelock, C. E., Cordonnier, C., Naka, H., Al-Shahi Salman, R., Sudlow, C. L., Edinburgh Stroke Study, G., Sorimachi, T., Werring, D. J., Gregoire, S. M., Imaizumi, T., Lee, S. H., Briley, D. & Rothwell, P. M. 2010. Antithrombotic drug use, cerebral microbleeds, and intracerebral hemorrhage: a systematic review of published and unpublished studies. *Stroke,* 41**,** 1222-8.

Maia, L. F., Vasconcelos, C., Seixas, S., Magalhaes, R. & Correia, M. 2006. Lobar brain hemorrhages and white matter changes: Clinical, radiological and laboratorial profiles. *Cerebrovasc Dis,* 22**,** 155-61.

Maia, L. F., Mackenzie, I. R. & Feldman, H. H. 2007. Clinical phenotypes of Cerebral Amyloid Angiopathy. *J Neurol Sci,* 257**,** 23-30.

Makin, S. D., Turpin, S., Dennis, M. S. & Wardlaw, J. M. 2013. Cognitive impairment after lacunar stroke: systematic review and meta-analysis of incidence, prevalence and comparison with other stroke subtypes. *J Neurol Neurosurg Psychiatry,* 84**,** 893-900.

Mandybur, T. I. 1975. The incidence of cerebral amyloid angiopathy in Alzheimer's disease. *Neurology,* 25**,** 120-6.

Mandybur, T. I. 1986. Cerebral amyloid angiopathy: the vascular pathology and complications. *J Neuropathol Exp Neurol,* 45**,** 79-90.

Mant, J., Hobbs, F. D., Fletcher, K., Roalfe, A., Fitzmaurice, D., Lip, G. Y., Murray, E., investigators, B. & Midland Research Practices, N. 2007. Warfarin versus aspirin for stroke prevention in an elderly community population with atrial fibrillation (the Birmingham Atrial Fibrillation Treatment of the Aged Study, BAFTA): a randomised controlled trial. *Lancet,* 370**,** 493-503.

Marin-Padilla, M. & Knopman, D. S. 2011. Developmental aspects of the intracerebral microvasculature and perivascular spaces: insights into brain response to late-life diseases. *J Neuropathol Exp Neurol,* 70**,** 1060-9.

Markus, H. S., Martin, R. J., Simpson, M. A., Dong, Y. B., Ali, N., Crosby, A. H. & Powell, J. F. 2002. Diagnostic strategies in CADASIL. *Neurology,* 59**,** 1134-8.

Martin, F. 1947. *Contribution à l'étude de l'amyloidose nodulaire isolée du coeur,* Zurich, Thèse de medicine.

Mathis, C. A., Wang, Y., Holt, D. P., Huang, G. F., Debnath, M. L. & Klunk, W. E. 2003. Synthesis and evaluation of 11C-labeled 6-substituted 2-arylbenzothiazoles as amyloid imaging agents. *J Med Chem,* 46**,** 2740-54.

Matsuoka, S. 1939. Studien uber Hirnblutung und- erweichung. (III Mitteilung). Uber kleine Aneurysmen in den Gehirnen ohne Blutung bezw. Blutunggsfreien Hirnpartien. *Trans Soc Pathol Jap.,* 29**,** 449-54.

Maxwell, S. S., Jackson, C. A., Paternoster, L., Cordonnier, C., Thijs, V., Al-Shahi Salman, R. & Sudlow, C. L. 2011. Genetic associations with brain microbleeds: Systematic review and meta-analyses. *Neurology,* 77**,** 158-67.

McAleese, K.E., Firbank, M., Hall, R., Neal, J.W., Mann, D.M., Esiri, M., Jellinger, K.A., O'Brien, J.T., Attems, J. 2012. Magnetic resonance imaging of post mortem brains reliably reflects subcortical vascular pathology of frontal, parietal and occipital white matter. Neuropathol Appl Neurobiol, 39, 485-97.

McCarron, M. O., Nicoll, J. A., Stewart, J., Ironside, J. W., Mann, D. M., Love, S., Graham, D. I. & Dewar, D. 1999. The apolipoprotein E epsilon2 allele and the pathological features in cerebral amyloid angiopathy-related hemorrhage. *J Neuropathol Exp Neurol,* 58**,** 711-8.

McCarron, M. O. & Nicoll, J. A. 2000. Apolipoprotein E genotype and cerebral amyloid angiopathy-related hemorrhage. *Ann N Y Acad Sci,* 903**,** 176-9.

McCarron, M. O. & Nicoll, J. A. 2004. Cerebral amyloid angiopathy and thrombolysis-related intracerebral haemorrhage. *Lancet Neurol,* 3**,** 484-92.

Menon, R. S. & Kidwell, C. S. 2009. Neuroimaging demonstration of evolving small vessel ischemic injury in cerebral amyloid angiopathy. *Stroke,* 40**,** e675-7.

Menon, R. S., Burgess, R. E., Wing, J. J., Gibbons, M. C., Shara, N. M., Fernandez, S., Jayam-Trouth, A., German, L., Sobotka, I., Edwards, D. & Kidwell, C. S. 2012. Predictors of highly prevalent brain ischemia in intracerebral hemorrhage. *Ann Neurol,* 71**,** 199-205.

Menzel, H. J., Kladetzky, R. G. & Assmann, G. 1983. Apolipoprotein E polymorphism and coronary artery disease. *Arteriosclerosis,* 3**,** 310-5.

Messori, A. & Salvolini, U. 2003. Postmortem MRI as a useful tool for investigation of cerebral microbleeds. *Stroke,* 34**,** 376-7; author reply 376-7.

Miklossy, J. 2003. Cerebral hypoperfusion induces cortical watershed microinfarcts which may further aggravate cognitive decline in Alzheimer's disease. *Neurol Res,* 25**,** 605-10.

Mittal, S., Wu, Z., Neelavalli, J. & Haacke, E. M. 2009. Susceptibility-weighted imaging: technical aspects and clinical applications, part 2. *AJNR Am J Neuroradiol,* 30**,** 232-52.

Miyao, S., Takano, A., Teramoto, J. & Takahashi, A. 1992. Leukoaraiosis in relation to prognosis for patients with lacunar infarction. *Stroke,* 23**,** 1434-8.

Miyasaka, Y., Barnes, M. E., Gersh, B. J., Cha, S. S., Bailey, K. R., Abhayaratna, W. P., Seward, J. B. & Tsang, T. S. 2006. Secular trends in incidence of atrial fibrillation in Olmsted County, Minnesota, 1980 to 2000, and implications on the projections for future prevalence. *Circulation,* 114**,** 119-25.

Mizutani, T., Kojima, H. & Miki, Y. 2000. Arterial dissections of penetrating cerebral arteries causing hypertension-induced cerebral hemorrhage. *J Neurosurg,* 93**,** 859-62.

Moreau, F., Patel, S., Lauzon, M. L., McCreary, C. R., Goyal, M., Frayne, R., Demchuk, A. M., Coutts, S. B. & Smith, E. E. 2012. Cavitation after acute symptomatic lacunar stroke depends on time, location, and MRI sequence. *Stroke,* 43**,** 1837-42.

Morgenstern, Z. I. 1935. Ein Fall von lokalem Amyloid der Hirngefäße. *Virchows Archiv* 294.

Morris, C. M., Candy, J. M., Keith, A. B., Oakley, A. E., Taylor, G. A., Pullen, R. G., Bloxham, C. A., Gocht, A. & Edwardson, J. A. 1992. Brain iron homeostasis. *J Inorg Biochem,* 47**,** 257-65.

Mungas, D., Jagust, W. J., Reed, B. R., Kramer, J. H., Weiner, M. W., Schuff, N., Norman, D., Mack, W. J., Willis, L. & Chui, H. C. 2001. MRI predictors of cognition in subcortical ischemic vascular disease and Alzheimer's disease. *Neurology,* 57**,** 2229-35.

Murphy, M. N. & Sima, A. A. 1985. Cerebral amyloid angiopathy associated with giant cell arteritis: a case report. *Stroke,* 16**,** 514-7.

Naka, H., Nomura, E., Wakabayashi, S., Kajikawa, H., Kohriyama, T., Mimori, Y., Nakamura, S. & Matsumoto, M. 2004. Frequency of asymptomatic microbleeds on T2*-weighted MR images of patients with recurrent stroke: association with combination of stroke subtypes and leukoaraiosis. *AJNR Am J Neuroradiol,* 25**,** 714-9.

Naka, H., Nomura, E., Takahashi, T., Wakabayashi, S., Mimori, Y., Kajikawa, H., Kohriyama, T. & Matsumoto, M. 2006. Combinations of the presence or absence of cerebral microbleeds and advanced white matter hyperintensity as predictors of subsequent stroke types. *AJNR Am J Neuroradiol,* 27**,** 830-5.

Naka, H., Nomura, E., Kitamura, J., Imamura, E., Wakabayashi, S. & Matsumoto, M. 2013. Antiplatelet therapy as a risk factor for microbleeds in intracerebral hemorrhage patients: analysis using specific antiplatelet agents. *J Stroke Cerebrovasc Dis,* 22**,** 834-40.

Nandigam, R. N., Viswanathan, A., Delgado, P., Skehan, M. E., Smith, E. E., Rosand, J., Greenberg, S. M. & Dickerson, B. C. 2009. MR imaging detection of cerebral microbleeds: effect of susceptibility-weighted imaging, section thickness, and field strength. *AJNR Am J Neuroradiol,* 30**,** 338-43.

Nelson, H. E. 1976. A modified card sorting test sensitive to frontal lobe defects. *Cortex,* 12**,** 313-24.

Neumann, M. A. 1960. Combined amyloid vascular changes and argyrophilic plaques in the central nervous system. *J Neuropathol Exp Neurol,* 19**,** 370-82.

Nighoghossian, N., Hermier, M., Adeleine, P., Blanc-Lasserre, K., Derex, L., Honnorat, J., Philippeau, F., Dugor, J. F., Froment, J. C. & Trouillas, P. 2002. Old microbleeds are a potential risk factor for cerebral bleeding after ischemic stroke: a gradient-echo T2*-weighted brain MRI study. *Stroke,* 33**,** 735-42.

Nishikawa, T., Ueba, T., Kajiwara, M., Fujisawa, I., Miyamatsu, N. & Yamashita, K. 2009. Cerebral microbleeds predict first-ever symptomatic cerebrovascular events. *Clin Neurol Neurosurg,* 111**,** 825-8.

Norrving, B. 2008. Lacunar infarcts: no black holes in the brain are benign. *Pract Neurol,* 8**,** 222-8.

O'Donnell, H. C., Rosand, J., Knudsen, K. A., Furie, K. L., Segal, A. Z., Chiu, R. I., Ikeda, D. & Greenberg, S. M. 2000. Apolipoprotein E genotype and the risk of recurrent lobar intracerebral hemorrhage. *N Engl J Med,* 342**,** 240-5.

O'Donnell, M. J., Xavier, D., Liu, L., Zhang, H., Chin, S. L., Rao-Melacini, P., Rangarajan, S., Islam, S., Pais, P., McQueen, M. J., Mondo, C., Damasceno, A., Lopez-Jaramillo, P., Hankey, G. J., Dans, A. L., Yusoff, K., Truelsen, T., Diener, H. C., Sacco, R. L., Ryglewicz, D., Czlonkowska, A., Weimar, C., Wang, X., Yusuf, S. & investigators, I. 2010. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study. *Lancet,* 376**,** 112-23.

O'Sullivan, M., Lythgoe, D. J., Pereira, A. C., Summers, P. E., Jarosz, J. M., Williams, S. C. & Markus, H. S. 2002. Patterns of cerebral blood flow reduction in patients with ischemic leukoaraiosis. *Neurology,* 59**,** 321-6.

O'Sullivan, M., Rich, P. M., Barrick, T. R., Clark, C. A. & Markus, H. S. 2003. Frequency of subclinical lacunar infarcts in ischemic leukoaraiosis and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *AJNR Am J Neuroradiol,* 24**,** 1348-54.

Offenbacher, H., Fazekas, F., Schmidt, R., Koch, M., Fazekas, G. & Kapeller, P. 1996. MR of cerebral abnormalities concomitant with primary intracerebral hematomas. *AJNR Am J Neuroradiol,* 17**,** 573-8.

Ogomori, K., Kitamoto, T., Tateishi, J., Sato, Y., Suetsugu, M. & Abe, M. 1989. Beta-protein amyloid is widely distributed in the central nervous system of patients with Alzheimer's disease. *Am J Pathol,* 134**,** 243-51.

Okazaki, H., Reagan, T. J. & Campbell, R. J. 1979. Clinicopathologic studies of primary cerebral amyloid angiopathy. *Mayo Clin Proc,* 54**,** 22-31.

Olichney, J. M., Hansen, L. A., Hofstetter, C. R., Grundman, M., Katzman, R. & Thal, L. J. 1995. Cerebral infarction in Alzheimer's disease is associated with severe amyloid angiopathy and hypertension. *Arch Neurol,* 52**,** 702-8.

Orken, D. N., Kenangil, G., Uysal, E. & Forta, H. 2009. Cerebral microbleeds in ischemic stroke patients on warfarin treatment. *Stroke,* 40**,** 3638-40.

Orken, D. N., Uysal, E., Timer, E., Kuloglu-Pazarci, N., Mumcu, S. & Forta, H. 2013. New cerebral microbleeds in ischemic stroke patients on warfarin treatment: Two-year follow-up. *Clin Neurol Neurosurg,* 115**,** 1682-5.

Ovbiagele, B., Saver, J. L., Sanossian, N., Salamon, N., Villablanca, P., Alger, J. R., Razinia, T., Kim, D. & Liebeskind, D. S. 2006. Predictors of cerebral microbleeds in acute ischemic stroke and TIA patients. *Cerebrovasc Dis,* 22**,** 378-83.

Ozturk, M. H. & Aydingoz, U. 2002. Comparison of MR signal intensities of cerebral perivascular (Virchow-Robin) and subarachnoid spaces. *J Comput Assist Tomogr,* 26**,** 902-4.

Pantelakis, S. 1954. Un type particulier d'angiopathie sénile du système nerveux central. *Mschr Psychiat Neurol,* 128**,** 219-256.

Pantoni, L. 2003. Not-so-silent infarcts. *Lancet Neurol,* 2**,** 335.

Pantoni, L. 2010. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol,* 9**,** 689-701.

Park, J. H., Park, S. W., Kang, S. H., Nam, T. K., Min, B. K. & Hwang, S. N. 2009. Detection of traumatic cerebral microbleeds by susceptibility-weighted image of MRI. *J Korean Neurosurg Soc,* 46**,** 365-9.

Park, J. H., Seo, S. W., Kim, C., Kim, G. H., Noh, H. J., Kim, S. T., Kwak, K. C., Yoon, U., Lee, J. M., Lee, J. W., Shin, J. S., Kim, C. H., Noh, Y., Cho, H., Kim, H. J., Yoon, C. W., Oh, S. J., Kim, J. S., Choe, Y. S., Lee, K. H., Lee, J. H., Ewers, M., Weiner, M. W., Werring, D. J. & Na, D. L. 2013. Pathogenesis of cerebral microbleeds: In vivo imaging of amyloid and subcortical ischemic small vessel disease in 226 individuals with cognitive impairment. *Ann Neurol,* 73**,** 584-93.

Pasquier, F. & Leys, D. 1997. Why are stroke patients prone to develop dementia? *J Neurol,* 244**,** 135-42.

Passero, S., Burgalassi, L., D'Andrea, P. & Battistini, N. 1995. Recurrence of bleeding in patients with primary intracerebral hemorrhage. *Stroke,* 26**,** 1189-92.

Pendlebury, S. T. & Rothwell, P. M. 2009. Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis. *Lancet Neurol,* 8**,** 1006-18.

Peters, R., Beckett, N., Forette, F., Tuomilehto, J., Clarke, R., Ritchie, C., Waldman, A., Walton, I., Poulter, R., Ma, S., Comsa, M., Burch, L., Fletcher, A. & Bulpitt, C. 2008. Incident dementia and blood pressure lowering in the Hypertension in the Very Elderly Trial cognitive function assessment (HYVET-COG): a double-blind, placebo controlled trial. *Lancet Neurol,* 7**,** 683-9.

Pettersen, J. A., Sathiyamoorthy, G., Gao, F. Q., Szilagyi, G., Nadkarni, N. K., St George-Hyslop, P., Rogaeva, E. & Black, S. E. 2008. Microbleed topography, leukoaraiosis, and cognition in probable Alzheimer disease from the Sunnybrook dementia study. *Arch Neurol,* 65**,** 790-5.

Pfeifer, L. A., White, L. R., Ross, G. W., Petrovitch, H. & Launer, L. J. 2002. Cerebral amyloid angiopathy and cognitive function: the HAAS autopsy study. *Neurology,* 58**,** 1629-34.

Piazza, F., Greenberg, S. M., Savoiardo, M., Gardinetti, M., Chiapparini, L., Raicher, I., Nitrini, R., Sakaguchi, H., Brioschi, M., Billo, G., Colombo, A., Lanzani, F., Piscosquito, G., Carriero, M. R., Giaccone, G., Tagliavini, F., Ferrarese, C. & Difrancesco, J. C. 2013. Anti-amyloid beta autoantibodies in cerebral amyloid angiopathy-related inflammation: Implications for Amyloid-Modifying Therapies. *Ann Neurol*.

Poels, M. M., Vernooij, M. W., Ikram, M. A., Hofman, A., Krestin, G. P., van der Lugt, A. & Breteler, M. M. 2010. Prevalence and risk factors of cerebral microbleeds: an update of the Rotterdam scan study. *Stroke,* 41**,** S103-6.

Poels, M. M., Ikram, M. A., van der Lugt, A., Hofman, A., Krestin, G. P., Breteler, M. M. & Vernooij, M. W. 2011. Incidence of cerebral microbleeds in the general population: the Rotterdam Scan Study. *Stroke,* 42**,** 656-61.

Poels, M. M., Ikram, M. A., van der Lugt, A., Hofman, A., Niessen, W. J., Krestin, G. P., Breteler, M. M. & Vernooij, M. W. 2012a. Cerebral microbleeds are associated with worse cognitive function: the Rotterdam Scan Study. *Neurology,* 78**,** 326-33.

Poels, M. M., Ikram, M. A. & Vernooij, M. W. 2012b. Improved MR imaging detection of cerebral microbleeds more accurately identifies persons with vasculopathy. *AJNR Am J Neuroradiol,* 33**,** 1553-6.

Poirier, J. & Derouesne, C. 1985. [The concept of cerebral lacunae from 1838 to the present]. *Rev Neurol (Paris),* 141**,** 3-17.

Potter, G. M., Doubal, F. N., Jackson, C. A., Chappell, F. M., Sudlow, C. L., Dennis, M. S. & Wardlaw, J. M. 2010. Counting cavitating lacunes underestimates the burden of lacunar infarction. *Stroke,* 41**,** 267-72.

Prabhakaran, S., Gupta, R., Ouyang, B., John, S., Temes, R. E., Mohammad, Y., Lee, V. H. & Bleck, T. P. 2010. Acute brain infarcts after spontaneous intracerebral hemorrhage: a diffusion-weighted imaging study. *Stroke,* 41**,** 89-94.

Prins, N. D., van Dijk, E. J., den Heijer, T., Vermeer, S. E., Koudstaal, P. J., Oudkerk, M., Hofman, A. & Breteler, M. M. 2004. Cerebral white matter lesions and the risk of dementia. *Arch Neurol,* 61**,** 1531-4.

Prins, N. D., van Dijk, E. J., den Heijer, T., Vermeer, S. E., Jolles, J., Koudstaal, P. J., Hofman, A. & Breteler, M. M. 2005. Cerebral small-vessel disease and decline in information processing speed, executive function and memory. *Brain,* 128**,** 2034-41.

Probst, A. & Ulrich, J. 1985. Amyloid angiopathy combined with granulomatous angiitis of the central nervous system: report on two patients. *Clin Neuropathol,* 4**,** 250-9.

Progress Collaborative Group 2001. Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6,105 individuals with previous stroke or transient ischaemic attack. *Lancet,* 358**,** 1033-41.

Puccio, D., Novo, G., Baiamonte, V., Nuccio, A., Fazio, G., Corrado, E., Coppola, G., Muratori, I., Vernuccio, L. & Novo, S. 2009. Atrial fibrillation and mild cognitive impairment: what correlation? *Minerva Cardioangiol,* 57**,** 143-50.

Purandare, N. 2009. Preventing dementia: role of vascular risk factors and cerebral emboli. *Br Med Bull,* 91**,** 49-59.

Qiu, C., Cotch, M. F., Sigurdsson, S., Jonsson, P. V., Jonsdottir, M. K., Sveinbjrnsdottir, S., Eiriksdottir, G., Klein, R., Harris, T. B., van Buchem, M. A., Gudnason, V. & Launer, L. J. 2010. Cerebral microbleeds, retinopathy, and dementia: the AGES-Reykjavik Study. *Neurology,* 75**,** 2221-8.

Rajan, S. S. 1998. *MRI : a conceptual overview,* New York, Springer.

Rannikmae, K., Samarasekera, N., Martinez-Gonzalez, N. A., Al-Shahi Salman, R. & Sudlow, C. L. 2013. Genetics of cerebral amyloid angiopathy: systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry,* 84**,** 901-8.

Raychaudhuri, R., Batjer, H. H. & Awad, I. A. 2005. Intracranial cavernous angioma: a practical review of clinical and biological aspects. *Surg Neurol,* 63**,** 319-28; discussion 328.

Reddy, D. J. 1951. Amyloidosis. *Ind Med Gaz,* 86**,** 548-51.

Regli, F., Vonsattel, J. P., Perentes, E. & Assal, G. 1981. [Cerebral amyloid angiopathy. A clinicopathological study (author's transl)]. *Rev Neurol (Paris),* 137**,** 181-94.

Reijmer, Y. D., Leemans, A., Brundel, M., Kappelle, L. J., Biessels, G. J. & Utrecht Vascular Cognitive Impairment Study, G. 2013. Disruption of the cerebral white matter network is related to slowing of information processing speed in patients with type 2 diabetes. *Diabetes,* 62**,** 2112-5.

Reinhard, M., Neunhoeffer, F., Gerds, T. A., Niesen, W. D., Buttler, K. J., Timmer, J., Schmidt, B., Czosnyka, M., Weiller, C. & Hetzel, A. 2010. Secondary decline of cerebral autoregulation is associated with worse outcome after intracerebral hemorrhage. *Intensive Care Med,* 36**,** 264-71.

Richardson, E. P., Jr. 1985. Amyloid in the human brain. *West J Med,* 143**,** 518-9.

Rigamonti, D., Drayer, B. P., Johnson, P. C., Hadley, M. N., Zabramski, J. & Spetzler, R. F. 1987. The MRI appearance of cavernous malformations (angiomas). *J Neurosurg,* 67**,** 518-24.

Rincon, F. & Wright, C. B. 2013. Vascular cognitive impairment. *Curr Opin Neurol,* 26**,** 29-36.

Roberts, T. P. & Mikulis, D. 2007. Neuro MR: principles. *J Magn Reson Imaging,* 26**,** 823-37.

Roch, J. A., Nighoghossian, N., Hermier, M., Cakmak, S., Picot, M., Honnorat, J., Derex, L. & Trouillas, P. 2005. Transient neurologic symptoms related to cerebral amyloid angiopathy: usefulness of T2*-weighted imaging. *Cerebrovasc Dis,* 20**,** 412-4.

Roher, A. E., Kuo, Y. M., Esh, C., Knebel, C., Weiss, N., Kalback, W., Luehrs, D. C., Childress, J. L., Beach, T. G., Weller, R. O. & Kokjohn, T. A. 2003. Cortical and leptomeningeal cerebrovascular amyloid and white matter pathology in Alzheimer's disease. *Mol Med,* 9**,** 112-22.

Rolfs, A., Fazekas, F., Grittner, U., Dichgans, M., Martus, P., Holzhausen, M., Bottcher, T., Heuschmann, P. U., Tatlisumak, T., Tanislav, C., Jungehulsing, G. J., Giese, A. K., Putaala, J., Huber, R., Bodechtel, U., Lichy, C., Enzinger, C., Schmidt, R., Hennerici, M. G., Kaps, M., Kessler, C., Lackner, K., Paschke, E., Meyer, W., Mascher, H., Riess, O., Kolodny, E., Norrving, B. & Stroke in Young Fabry Patients, I. 2013. Acute cerebrovascular disease in the young: the Stroke in Young Fabry Patients study. *Stroke,* 44**,** 340-9.

Román, G. C. 2002. On the history of lacunes, etat crible, and the white matter lesions of vascular dementia. *Cerebrovasc Dis,* 13 Suppl 2**,** 1-6.

Roob, G., Kleinert, R., Seifert, T., Lechner, A., Kapeller, P., Kleinert, G., Schmidt, R., Fazekas, F. & Hartung, H. P. 1999a. [Indications of cerebral micro-hemorrhage in MRI. Comparative histological findings and possible clinical significance]. *Nervenarzt,* 70**,** 1082-7.

Roob, G., Schmidt, R., Kapeller, P., Lechner, A., Hartung, H. P. & Fazekas, F. 1999b. MRI evidence of past cerebral microbleeds in a healthy elderly population. *Neurology,* 52**,** 991-4.

Roob, G. & Fazekas, F. 2000. Magnetic resonance imaging of cerebral microbleeds. *Curr Opin Neurol,* 13**,** 69-73.

Roob, G., Lechner, A., Schmidt, R., Flooh, E., Hartung, H. P. & Fazekas, F. 2000. Frequency and location of microbleeds in patients with primary intracerebral hemorrhage. *Stroke,* 31**,** 2665-9.

Rosand, J., Hylek, E. M., O'Donnell, H. C. & Greenberg, S. M. 2000. Warfarin-associated hemorrhage and cerebral amyloid angiopathy: a genetic and pathologic study. *Neurology,* 55**,** 947-51.

Rosand, J., Eckman, M. H., Knudsen, K. A., Singer, D. E. & Greenberg, S. M. 2004. The effect of warfarin and intensity of anticoagulation on outcome of intracerebral hemorrhage. *Arch Intern Med,* 164**,** 880-4.

Rosand, J., Muzikansky, A., Kumar, A., Wisco, J. J., Smith, E. E., Betensky, R. A. & Greenberg, S. M. 2005. Spatial clustering of hemorrhages in probable cerebral amyloid angiopathy. *Ann Neurol,* 58**,** 459-62.

Rosenberg, G. A. 2009. Inflammation and white matter damage in vascular cognitive impairment. *Stroke,* 40**,** S20-3.

Rosenblum, W. I. 2003. Cerebral hemorrhage produced by ruptured dissecting aneurysm in miliary aneurysm. *Ann Neurol,* 54**,** 376-8.

Ross Russell, R. W. 1963. Observations On Intracerebral Aneurysms. *Brain,* 86**,** 425-42.

Rouhl, R. P., van Oostenbrugge, R. J., Damoiseaux, J. G., Debrus-Palmans, L. L., Theunissen, R. O., Knottnerus, I. L., Staals, J. E., Delanghe, J. R., Tervaert, J. W. & Lodder, J. 2009. Haptoglobin phenotype may alter endothelial progenitor cell cluster formation in cerebral small vessel disease. *Curr Neurovasc Res,* 6**,** 32-41.

Sabri, O., Ringelstein, E. B., Hellwig, D., Schneider, R., Schreckenberger, M., Kaiser, H. J., Mull, M. & Buell, U. 1999. Neuropsychological impairment correlates with hypoperfusion and hypometabolism but not with severity of white matter lesions on MRI in patients with cerebral microangiopathy. *Stroke,* 30**,** 556-66.

Saczynski, J. S., Sigurdsson, S., Jonsdottir, M. K., Eiriksdottir, G., Jonsson, P. V., Garcia, M. E., Kjartansson, O., Lopez, O., van Buchem, M. A., Gudnason, V. & Launer, L. J. 2009. Cerebral infarcts and cognitive performance: importance of location and number of infarcts. *Stroke,* 40**,** 677-82.

Saini, M., Ikram, K., Hilal, S., Qiu, A., Venketasubramanian, N. & Chen, C. 2012. Silent stroke: not listened to rather than silent. *Stroke,* 43**,** 3102-4.

Saloheimo, P., Ahonen, M., Juvela, S., Pyhtinen, J., Savolainen, E. R. & Hillbom, M. 2006. Regular aspirin-use preceding the onset of primary intracerebral hemorrhage is an independent predictor for death. *Stroke,* 37**,** 129-33.

Samuelsson, M., Soderfeldt, B. & Olsson, G. B. 1996. Functional outcome in patients with lacunar infarction. *Stroke,* 27**,** 842-6.

Santos, M., Gold, G., Kovari, E., Herrmann, F. R., Bozikas, V. P., Bouras, C. & Giannakopoulos, P. 2009. Differential impact of lacunes and microvascular lesions on poststroke depression. *Stroke,* 40**,** 3557-62.

Savva, G. M., Wharton, S. B., Ince, P. G., Forster, G., Matthews, F. E., Brayne, C., Medical Research Council Cognitive, F. & Ageing, S. 2009. Age, neuropathology, and dementia. *N Engl J Med,* 360**,** 2302-9.

Saxena, R. & Koudstaal, P. 2004. Anticoagulants versus antiplatelet therapy for preventing stroke in patients with nonrheumatic atrial fibrillation and a history of stroke or transient ischemic attack. *Cochrane Database Syst Rev***,** CD000187.

Scharf, J., Brauherr, E., Forsting, M. & Sartor, K. 1994. Significance of haemorrhagic lacunes on MRI in patients with hypertensive cerebrovascular disease and intracerebral haemorrhage. *Neuroradiology,* 36**,** 504-8.

Scheid, R., Ott, D. V., Roth, H., Schroeter, M. L. & von Cramon, D. Y. 2007. Comparative magnetic resonance imaging at 1.5 and 3 Tesla for the evaluation of traumatic microbleeds. *J Neurotrauma,* 24**,** 1811-6.

Scheltens, P., Barkhof, F., Leys, D., Pruvo, J.P., Nauta, J.J., Vermersch, P., Steinling, M., Valk, J. 1993. A semiquantative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging. J Neurol Sci., 114:7-12.

Scheltens, P., Erkinjunti, T., Leys, D., Wahlund, L. O., Inzitari, D., del Ser, T., Pasquier, F., Barkhof, F., Mantyla, R., Bowler, J., Wallin, A., Ghika, J., Fazekas, F. & Pantoni, L.

1998. White matter changes on CT and MRI: an overview of visual rating scales. European Task Force on Age-Related White Matter Changes. *Eur Neurol,* 39**,** 80-9.

Schenck, J. F. 2003. Magnetic resonance imaging of brain iron. *J Neurol Sci,* 207**,** 99-102.

Schenck, J. F. & Zimmerman, E. A. 2004. High-field magnetic resonance imaging of brain iron: birth of a biomarker? *NMR Biomed,* 17**,** 433-45.

Scherzinger, A. L. & Hendee, W. R. 1985. Basic principles of magnetic resonance imaging--an update. *West J Med,* 143**,** 782-92.

Schlote, W. 1965. Amyloidnatur der Kongophilen, drusigen Entartung der Hirnarterien (Scholz) in Senium. *Acta Neuropathol (Berl),* 4**,** 449-468.

Schmidt, R., Grazer, A., Enzinger, C., Ropele, S., Homayoon, N., Pluta-Fuerst, A., Schwingenschuh, P., Katschnig, P., Cavalieri, M., Schmidt, H., Langkammer, C., Ebner, F. & Fazekas, F. 2011. MRI-detected white matter lesions: do they really matter? *J Neural Transm,* 118**,** 673-81.

Schneider, J. A., Wilson, R. S., Cochran, E. J., Bienias, J. L., Arnold, S. E., Evans, D. A. & Bennett, D. A. 2003. Relation of cerebral infarctions to dementia and cognitive function in older persons. *Neurology,* 60**,** 1082-8.

Schneider, J. A. 2007. Brain microbleeds and cognitive function. *Stroke,* 38**,** 1730-1.

Schneider, J. A., Arvanitakis, Z., Bang, W. & Bennett, D. A. 2007. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology,* 69**,** 2197-204.

Scholz, W. 1938. Studien zur pathologie der Hirngefabell: Die drusige Entartung der Hirnarterien und capillaren. *Gesamte Neurol Psychiat* 162**,** 694-715.

Schrag, M., McAuley, G., Pomakian, J., Jiffry, A., Tung, S., Mueller, C., Vinters, H. V., Haacke, E. M., Holshouser, B., Kido, D. & Kirsch, W. M. 2010. Correlation of hypointensities in susceptibility-weighted images to tissue histology in dementia patients with cerebral amyloid angiopathy: a postmortem MRI study. *Acta Neuropathol,* 119**,** 291-302.

Schreiber, S., Bueche, C. Z., Garz, C. & Braun, H. 2013. Blood brain barrier breakdown as the starting point of cerebral small vessel disease? - New insights from a rat model. *Exp Transl Stroke Med,* 5**,** 4.

Schwarz, P. 1970. *Amyloidosis: cause and manifestation of senile deterioration,* Springfield, Illinois, Charles C Thomas.

Seghier, M. L., Kolanko, M. A., Leff, A. P., Jager, H. R., Gregoire, S. M. & Werring, D. J. 2011. Microbleed detection using automated segmentation (MIDAS): a new method applicable to standard clinical MR images. *PLoS One,* 6**,** e17547.

Sehgal, V., Delproposto, Z., Haacke, E. M., Tong, K. A., Wycliffe, N., Kido, D. K., Xu, Y., Neelavalli, J., Haddar, D. & Reichenbach, J. R. 2005. Clinical applications of

neuroimaging with susceptibility-weighted imaging. *J Magn Reson Imaging,* 22**,** 439-50.

Seo, S. W., Hwa Lee, B., Kim, E. J., Chin, J., Sun Cho, Y., Yoon, U. & Na, D. L. 2007. Clinical significance of microbleeds in subcortical vascular dementia. *Stroke,* 38**,** 1949-51.

Seshadri, S., Beiser, A., Kelly-Hayes, M., Kase, C. S., Au, R., Kannel, W. B. & Wolf, P. A. 2006. The lifetime risk of stroke: estimates from the Framingham Study. *Stroke,* 37**,** 345-50.

Shintaku, M., Osawa, K., Toki, J., Maeda, R. & Nishiyama, T. 1986. A case of granulomatous angiitis of the central nervous system associated with amyloid angiopathy. *Acta Neuropathol,* 70**,** 340-2.

Shoamanesh, A., Kwok, C. S. & Benavente, O. 2011. Cerebral microbleeds: histopathological correlation of neuroimaging. *Cerebrovasc Dis,* 32**,** 528-34.

Shoamanesh, A., Catanese, L., Sakai, O., Pikula, A. & Kase, C. S. 2013. Diffusion-weighted imaging hyperintensities in intracerebral hemorrhage: microinfarcts or microbleeds? *Ann Neurol,* 73**,** 795-6.

Simpson, J. E., Fernando, M. S., Clark, L., Ince, P. G., Matthews, F., Forster, G., O'Brien, J. T., Barber, R., Kalaria, R. N., Brayne, C., Shaw, P. J., Lewis, C. E., Wharton, S. B., Function, M. R. C. C. & Ageing Neuropathology Study, G. 2007. White matter lesions in an unselected cohort of the elderly: astrocytic, microglial and oligodendrocyte precursor cell responses. *Neuropathol Appl Neurobiol,* 33**,** 410-9.

Singh Alg, V. & Werring, D. J. 2011. Historical overview. *In:* Werring, D. J. (ed.) *Cerebral microbleeds: Pathophysiological to clinical practice.* Cambridge University Press.

Smith, D. B., Hitchcock, M. & Philpott, P. J. 1985. Cerebral amyloid angiopathy presenting as transient ischemic attacks. Case report. *J Neurosurg,* 63**,** 963-4.

Smith, E. E., Rosand, J., Knudsen, K. A., Hylek, E. M. & Greenberg, S. M. 2002. Leukoaraiosis is associated with warfarin-related hemorrhage following ischemic stroke. *Neurology,* 59**,** 193-7.

Smith, E. E. & Greenberg, S. M. 2009. Beta-amyloid, blood vessels, and brain function. *Stroke,* 40**,** 2601-6.

Smith, E. E. 2010. Leukoaraiosis and stroke. *Stroke,* 41**,** S139-43.

Smith, E. E., Nandigam, K. R., Chen, Y. W., Jeng, J., Salat, D., Halpin, A., Frosch, M., Wendell, L., Fazen, L., Rosand, J., Viswanathan, A. & Greenberg, S. M. 2010. MRI markers of small vessel disease in lobar and deep hemispheric intracerebral hemorrhage. *Stroke,* 41**,** 1933-8.

Snaphaan, L. & de Leeuw, F. E. 2007. Poststroke memory function in nondemented patients: a systematic review on frequency and neuroimaging correlates. *Stroke,* 38**,** 198-203.

Snowdon, D. A., Greiner, L. H., Mortimer, J. A., Riley, K. P., Greiner, P. A. & Markesbery, W. R. 1997. Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. *JAMA,* 277**,** 813-7.

Soo, Y. O., Yang, S. R., Lam, W. W., Wong, A., Fan, Y. H., Leung, H. H., Chan, A. Y., Leung, C., Leung, T. W. & Wong, L. K. 2008. Risk vs benefit of anti-thrombotic therapy in ischaemic stroke patients with cerebral microbleeds. *J Neurol,* 255**,** 1679-86.

Soo, Y. O. & Wong, L. K. 2011. Cerebral microbleeds and antithrombotoc treatment. *In:* Werring, D. J. (ed.) *Cerebral microbleeds: Pathophysiological to clinical practice.* New York: Cambridge University Press.

Soontornniyomkij, V., Lynch, M. D., Mermash, S., Pomakian, J., Badkoobehi, H., Clare, R. & Vinters, H. V. 2010. Cerebral microinfarcts associated with severe cerebral beta-amyloid angiopathy. *Brain Pathol,* 20**,** 459-67.

Spangler, K. M., Challa, V. R., Moody, D. M. & Bell, M. A. 1994. Arteriolar tortuosity of the white matter in aging and hypertension. A microradiographic study. *J Neuropathol Exp Neurol,* 53**,** 22-6.

Sperling, R., Salloway, S., Brooks, D. J., Tampieri, D., Barakos, J., Fox, N. C., Raskind, M., Sabbagh, M., Honig, L. S., Porsteinsson, A. P., Lieberburg, I., Arrighi, H. M., Morris, K. A., Lu, Y., Liu, E., Gregg, K. M., Brashear, H. R., Kinney, G. G., Black, R. & Grundman, M. 2012. Amyloid-related imaging abnormalities in patients with Alzheimer's disease treated with bapineuzumab: a retrospective analysis. *Lancet Neurol,* 11**,** 241-9.

Spreen, O. & Benton, A. L. 1969. *Neurosensory Center Comprehensive Examination for Aphasia. ,* Victoria, BC, Neuropsychology Laboratory, University of Victoria.

Staals, J., van Oostenbrugge, R. J., Knottnerus, I. L., Rouhl, R. P., Henskens, L. H. & Lodder, J. 2009. Brain microbleeds relate to higher ambulatory blood pressure levels in first-ever lacunar stroke patients. *Stroke,* 40**,** 3264-8.

Staekenborg, S. S., Koedam, E. L., Henneman, W. J., Stokman, P., Barkhof, F., Scheltens, P. & van der Flier, W. M. 2009. Progression of mild cognitive impairment to dementia: contribution of cerebrovascular disease compared with medial temporal lobe atrophy. *Stroke,* 40**,** 1269-74.

Stankiewicz, J., Panter, S. S., Neema, M., Arora, A., Batt, C. E. & Bakshi, R. 2007. Iron in chronic brain disorders: imaging and neurotherapeutic implications. *Neurotherapeutics,* 4**,** 371-86.

Stark, D. D. & Bradley, W. G. 1999. *Magnetic resonance imaging* St. Louis

London, Mosby.

Sudlow, C., Martinez Gonzalez, N. A., Kim, J. & Clark, C. 2006. Does apolipoprotein E genotype influence the risk of ischemic stroke, intracerebral hemorrhage, or subarachnoid hemorrhage? Systematic review and meta-analyses of 31 studies among 5961 cases and 17,965 controls. *Stroke,* 37**,** 364-70.

Sun, J., Soo, Y. O., Lam, W. W., Wong, K. S., Zeng, J. S. & Fan, Y. H. 2009. Different distribution patterns of cerebral microbleeds in acute ischemic stroke patients with and without hypertension. *Eur Neurol,* 62**,** 298-303.

Surbeck, K. B. 1961. *L'angiopathie dyshorique (Morel) de l'écorce cérébrale. Etude anatomo-clinique et statistique. Aspect génétique.* PhD, University of Geneva.

Sveinbjornsdottir, S., Sigurdsson, S., Aspelund, T., Kjartansson, O., Eiriksdottir, G., Valtysdottir, B., Lopez, O. L., van Buchem, M. A., Jonsson, P. V., Gudnason, V. & Launer, L. J. 2008. Cerebral microbleeds in the population based AGES-Reykjavik study: prevalence and location. *J Neurol Neurosurg Psychiatry,* 79**,** 1002-6.

Takebayashi, S. & Kaneko, M. 1983. Electron microscopic studies of ruptured arteries in hypertensive intracerebral hemorrhage. *Stroke,* 14**,** 28-36.

Tanaka, A., Ueno, Y., Nakayama, Y., Takano, K. & Takebayashi, S. 1999. Small chronic hemorrhages and ischemic lesions in association with spontaneous intracerebral hematomas. *Stroke,* 30**,** 1637-42.

Tanaka, M., Sakaguchi, M., Miwa, K. & Kitagawa, K. 2012. Cerebral microbleeds in patients with moyamoya-like vessels secondary to atherosclerosis. *Intern Med,* 51**,** 167-72.

Tang, W. K., Chen, Y. K., Lu, J. Y., Mok, V. C., Xiang, Y. T., Ungvari, G. S., Ahuja, A. T. & Wong, K. S. 2009. Microbleeds and post-stroke emotional lability. *J Neurol Neurosurg Psychiatry,* 80**,** 1082-6.

Tatsumi, S., Ayaki, T., Shinohara, M. & Yamamoto, T. 2008a. Type of gradient recalled-echo sequence results in size and number change of cerebral microbleeds. *AJNR Am J Neuroradiol,* 29**,** e13.

Tatsumi, S., Shinohara, M. & Yamamoto, T. 2008b. Direct comparison of histology of microbleeds with postmortem MR images: a case report. *Cerebrovasc Dis,* 26**,** 142-6.

Teo, J. T. H., Ramadan, H., Gregoire, S. M., Mufti, S., Lipman, G., Jäger, R. H., Losseff, N. & Werring, D. J. 2011. Can cerebral micronleeds cause an acute stroke syndrome. *Neurology Clinical Practice,* 1**,** 75-77.

Terry, R. D., Gonatas, N. K. & Weiss, M. 1964. Ultrastructural studies in Alzheimer's presenile dementia. *Am J Pathol,* 44**,** 269-297.

Thal, D. R., Ghebremedhin, E., Rub, U., Yamaguchi, H., Del Tredici, K. & Braak, H. 2002. Two types of sporadic cerebral amyloid angiopathy. *J Neuropathol Exp Neurol,* 61**,** 282-93.

Thal, D. R., Grinberg, L. T. & Attems, J. 2012. Vascular dementia: different forms of vessel disorders contribute to the development of dementia in the elderly brain. *Exp Gerontol,* 47**,** 816-24.

Thijs, V., Lemmens, R., Schoofs, C., Gorner, A., Van Damme, P., Schrooten, M. & Demaerel, P. 2010. Microbleeds and the risk of recurrent stroke. *Stroke,* 41**,** 2005-9.

Thomas, B., Somasundaram, S., Thamburaj, K., Kesavadas, C., Gupta, A. K., Bodhey, N. K. & Kapilamoorthy, T. R. 2008. Clinical applications of susceptibility weighted MR imaging of the brain - a pictorial review. *Neuroradiology,* 50**,** 105-16.

Torack, R. M. 1975. Congophilic angiopathy complicated by surgery and massive hemorrhage. A light and electron microscopic study. *Am J Pathol,* 81**,** 349-66.

Tournier-Lasserve, E., Joutel, A., Melki, J., Weissenbach, J., Lathrop, G. M., Chabriat, H., Mas, J. L., Cabanis, E. A., Baudrimont, M., Maciazek, J. & et al. 1993. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy maps to chromosome 19q12. *Nat Genet,* 3**,** 256-9.

Trennary, M. R., Crossen, B., DeBoe, J. & Leber, W. R. 1989. *Stroop Neuropsychological Screening Test (SNST)* Odessa, FL Psychological Assessment Resources.

Tress, B. M. & Brant-Zawadski, M. 1985. Nuclear magnetic resonance imaging. Basic principles. *Med J Aust,* 142**,** 21-4.

Tsushima, Y., Aoki, J. & Endo, K. 2003. Brain microhemorrhages detected on T2*-weighted gradient-echo MR images. *AJNR Am J Neuroradiol,* 24**,** 88-96.

Tsushima, Y. & Endo, K. 2006. Hypointensities in the brain on T2*-weighted gradient-echo magnetic resonance imaging. *Curr Probl Diagn Radiol,* 35**,** 140-50.

Tzourio, C., Anderson, C., Chapman, N., Woodward, M., Neal, B., MacMahon, S. & Chalmers, J. 2003. Effects of blood pressure lowering with perindopril and indapamide therapy on dementia and cognitive decline in patients with cerebrovascular disease. *Arch Intern Med,* 163**,** 1069-75.

Tzourio, C., Arima, H., Harrap, S., Anderson, C., Godin, O., Woodward, M., Neal, B., Bousser, M. G., Chalmers, J., Cambien, F. & MacMahon, S. 2008. APOE genotype, ethnicity, and the risk of cerebral hemorrhage. *Neurology,* 70**,** 1322-8.

U.S. Army 1944. *Army Individual Test Battery - Manual and directions for scoring,* Washington, DC, War Department, Adjunct General's Office.

Ueno, H., Naka, H., Ohshita, T., Kondo, K., Nomura, E., Ohtsuki, T., Kohriyama, T., Wakabayashi, S. & Matsumoto, M. 2008. Association between cerebral microbleeds on T2*-weighted MR images and recurrent hemorrhagic stroke in patients treated with warfarin following ischemic stroke. *AJNR Am J Neuroradiol,* 29**,** 1483-6.

Vahedi, K., Boukobza, M., Massin, P., Gould, D. B., Tournier-Lasserve, E. & Bousser, M. G. 2007. Clinical and brain MRI follow-up study of a family with COL4A1 mutation. *Neurology,* 69**,** 1564-8.

Valdés Hernández, M. del C., Morris, Z., Dickie, D.A., Royle, N.A., Muñoz Maniega, S., Aribisala, B.S., Bastin, M.E., Deary, I.J., Wardlaw, J.M. 2013. Close correlation between quantitative and qualitative assessments of white matter lesions. *Neuroepidemiology*, 40, 13-22.

van Beijnum, J., Lovelock, C. E., Cordonnier, C., Rothwell, P. M., Klijn, C. J., Al-Shahi Salman, R., Committee, S. S. & the Oxford Vascular, S. 2009. Outcome after spontaneous and arteriovenous malformation-related intracerebral haemorrhage: population-based studies. *Brain,* 132**,** 537-43.

van den Boom, R., Lesnik Oberstein, S. A., Ferrari, M. D., Haan, J. & van Buchem, M. A. 2003. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: MR imaging findings at different ages--3rd-6th decades. *Radiology,* 229**,** 683-90.

van den Boom, R., Bornebroek, M., Behloul, F., van den Berg-Huysmans, A. A., Haan, J. & van Buchem, M. A. 2005. Microbleeds in hereditary cerebral hemorrhage with amyloidosis-Dutch type. *Neurology,* 64**,** 1288-9.

van Dijk, E. J., Prins, N. D., Vermeer, S. E., Hofman, A., van Duijn, C. M., Koudstaal, P. J. & Breteler, M. M. 2004. Plasma amyloid beta, apolipoprotein E, lacunar infarcts, and white matter lesions. *Ann Neurol,* 55**,** 570-5.

van Dijk, E. J., Prins, N. D., Vrooman, H. A., Hofman, A., Koudstaal, P. J. & Breteler, M. M. 2008. Progression of cerebral small vessel disease in relation to risk factors and cognitive consequences: Rotterdam Scan study. *Stroke,* 39**,** 2712-9.

van Es, A. C., van der Grond, J., de Craen, A. J., Admiraal-Behloul, F., Blauw, G. J. & van Buchem, M. A. 2008. Risk factors for cerebral microbleeds in the elderly. *Cerebrovasc Dis,* 26**,** 397-403.

van Norden, A. G., van den Berg, H. A., de Laat, K. F., Gons, R. A., van Dijk, E. J. & de Leeuw, F. E. 2011. Frontal and temporal microbleeds are related to cognitive function: the Radboud University Nijmegen Diffusion Tensor and Magnetic Resonance Cohort (RUN DMC) Study. *Stroke,* 42**,** 3382-6.

van Rooden, S., van der Grond, J., van den Boom, R., Haan, J., Linn, J., Greenberg, S. M. & van Buchem, M. A. 2009. Descriptive analysis of the Boston criteria applied to a Dutch-type cerebral amyloid angiopathy population. *Stroke,* 40**,** 3022-7.

van Straaten, E.C. , Fazekas, F., Rostrup, E. et al. 2006. Impact of white matter hyperintensities scoring method on correlations with clinical data: the LADIS study. *Stroke*, 37, 836-40.

van Swieten, J. C., Staal, S., Kappelle, L. J., Derix, M. M. & van Gijn, J. 1996. Are white matter lesions directly associated with cognitive impairment in patients with lacunar infarcts? *J Neurol,* 243**,** 196-200.

van Walraven, C., Hart, R. G., Singer, D. E., Laupacis, A., Connolly, S., Petersen, P., Koudstaal, P. J., Chang, Y. & Hellemons, B. 2002. Oral anticoagulants vs aspirin in nonvalvular atrial fibrillation: an individual patient meta-analysis. *JAMA,* 288**,** 2441-8.

Vandenbroucke, J. P. 2011. Why do the results of randomised and observational studies differ? *BMJ,* 343**,** d7020.

Veglio, F., Paglieri, C., Rabbia, F., Bisbocci, D., Bergui, M. & Cerrato, P. 2009. Hypertension and cerebrovascular damage. *Atherosclerosis,* 205**,** 331-41.

Verghese, P. B., Castellano, J. M. & Holtzman, D. M. 2011. Apolipoprotein E in Alzheimer's disease and other neurological disorders. *Lancet Neurol,* 10**,** 241-52.

Vermeer, S. E., Prins, N. D., den Heijer, T., Hofman, A., Koudstaal, P. J. & Breteler, M. M. 2003a. Silent brain infarcts and the risk of dementia and cognitive decline. *N Engl J Med,* 348**,** 1215-22.

Vermeer, S. E., Den Heijer, T., Koudstaal, P. J., Oudkerk, M., Hofman, A., Breteler, M. M. & Rotterdam Scan, S. 2003b. Incidence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. *Stroke,* 34**,** 392-6.

Vermeer, S. E., Longstreth, W. T., Jr. & Koudstaal, P. J. 2007. Silent brain infarcts: a systematic review. *Lancet Neurol,* 6**,** 611-9.

Vernooij, M. W., Ikram, M. A., Wielopolski, P. A., Krestin, G. P., Breteler, M. M. & van der Lugt, A. 2008a. Cerebral microbleeds: accelerated 3D T2*-weighted GRE MR imaging versus conventional 2D T2*-weighted GRE MR imaging for detection. *Radiology,* 248**,** 272-7.

Vernooij, M. W., van der Lugt, A., Ikram, M. A., Wielopolski, P. A., Niessen, W. J., Hofman, A., Krestin, G. P. & Breteler, M. M. 2008b. Prevalence and risk factors of cerebral microbleeds: the Rotterdam Scan Study. *Neurology,* 70**,** 1208-14.

Vernooij, M. W., Haag, M. D., van der Lugt, A., Hofman, A., Krestin, G. P., Stricker, B. H. & Breteler, M. M. 2009. Use of antithrombotic drugs and the presence of cerebral microbleeds: the Rotterdam Scan Study. *Arch Neurol,* 66**,** 714-20.

Vinters, H. V. & Gilbert, J. J. 1983. Cerebral amyloid angiopathy: incidence and complications in the aging brain. II. The distribution of amyloid vascular changes. *Stroke,* 14**,** 924-8.

Vinters, H. V. 1987. Cerebral amyloid angiopathy. A critical review. *Stroke,* 18**,** 311-24.

Viswanathan, A., Rakich, S. M., Engel, C., Snider, R., Rosand, J., Greenberg, S. M. & Smith, E. E. 2006. Antiplatelet use after intracerebral hemorrhage. *Neurology,* 66**,** 206-9.

Viswanathan, A. & Chabriat, H. 2006. Cerebral microhemorrhage. *Stroke,* 37**,** 550-5.

Viswanathan, A., Gschwendtner, A., Guichard, J. P., Buffon, F., Cumurciuc, R., O'Sullivan, M., Holtmannspotter, M., Pachai, C., Bousser, M. G., Dichgans, M. & Chabriat, H. 2007. Lacunar lesions are independently associated with disability and cognitive impairment in CADASIL. *Neurology,* 69**,** 172-9.

Viswanathan, A., Patel, P., Rahman, R., Nandigam, R. N., Kinnecom, C., Bracoud, L., Rosand, J., Chabriat, H., Greenberg, S. M. & Smith, E. E. 2008. Tissue microstructural changes are independently associated with cognitive impairment in cerebral amyloid angiopathy. *Stroke,* 39**,** 1988-92.

Viswanathan, A., Godin, O., Jouvent, E., O'Sullivan, M., Gschwendtner, A., Peters, N., Duering, M., Guichard, J. P., Holtmannspotter, M., Dufouil, C., Pachai, C., Bousser, M. G., Dichgans, M. & Chabriat, H. 2010. Impact of MRI markers in subcortical vascular dementia: a multi-modal analysis in CADASIL. *Neurobiol Aging,* 31**,** 1629-36.

Viswanathan, A., Chabriat, H. & Dichgans, M. 2011. Cerebral microbleeds in CADASIL. *In:* Werring, D. J. (ed.) *Cerebral microbleeds: Pathophysiological to clinical practice.* New York: Cambridge University Press.

Vonsattel, J. P., Myers, R. H., Hedley-Whyte, E. T., Ropper, A. H., Bird, E. D. & Richardson, E. P., Jr. 1991. Cerebral amyloid angiopathy without and with cerebral hemorrhages: a comparative histological study. *Ann Neurol,* 30**,** 637-49.

Wahlund, L. O., Barkhof, F., Fazekas, F., Bronge, L., Augustin, M., Sjogren, M., Wallin, A., Ader, H., Leys, D., Pantoni, L., Pasquier, F., Erkinjuntti, T. & Scheltens, P. 2001. A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke,* 32**,** 1318-22.

Wakai, S. & Nagai, M. 1989. Histological verification of microaneurysms as a cause of cerebral haemorrhage in surgical specimens. *J Neurol Neurosurg Psychiatry,* 52**,** 595-9.

Wardlaw, J. M., Sandercock, P. A., Dennis, M. S. & Starr, J. 2003. Is breakdown of the blood-brain barrier responsible for lacunar stroke, leukoaraiosis, and dementia? *Stroke,* 34**,** 806-12.

Wardlaw, J. M. 2005. What causes lacunar stroke? *J Neurol Neurosurg Psychiatry,* 76**,** 617-9.

Wardlaw, J. M., Doubal, F., Armitage, P., Chappell, F., Carpenter, T., Munoz Maniega, S., Farrall, A., Sudlow, C., Dennis, M. & Dhillon, B. 2009. Lacunar stroke is associated with diffuse blood-brain barrier dysfunction. *Ann Neurol,* 65**,** 194-202.

Wardlaw, J. M. 2011. Imaging of lacunar stroke and related small vessel disease phenotypes. Relationship to background risk factors and future disease risk. . *European Stroke Conference.* Hamburg.

Wardlaw, J. M., Smith, E. E., Biessels, G. J., Cordonnier, C., Fazekas, F., Frayne, R., Lindley, R. I., O'Brien, J. T., Barkhof, F., Benavente, O. R., Black, S. E., Brayne, C., Breteler, M., Chabriat, H., Decarli, C., de Leeuw, F. E., Doubal, F., Duering, M., Fox, N. C., Greenberg, S., Hachinski, V., Kilimann, I., Mok, V., Oostenbrugge, R., Pantoni, L., Speck, O., Stephan, B. C., Teipel, S., Viswanathan, A., Werring, D., Chen, C., Smith, C., van Buchem, M., Norrving, B., Gorelick, P. B., Dichgans, M. & nEuroimaging, S. T. f. R. V. c. o. 2013. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol,* 12**,** 822-38.

Warlow, C. P., van Gijn, J., Dennis, M. S., Wardlaw, J. M., Bamford, J. M., Hankey, G. J., Sandercock, P. A. G., Rinkel, G., Langhorne, P., Sudlow, C. & Rothwell, P. 2008. *Stroke: A practical guide to management*, Wiley-Blackwell.

Watanabe, A. & Kobashi, T. 2005. Lateral gaze disturbance due to cerebral microbleed in the medial lemniscus in the mid-pontine region: a case report. *Neuroradiology,* 47**,** 908-11.

Wattendorff, A. R., Frangione, B., Luyendijk, W. & Bots, G. T. 1995. Hereditary cerebral haemorrhage with amyloidosis, Dutch type (HCHWA-D): clinicopathological studies. *J Neurol Neurosurg Psychiatry,* 58**,** 699-705.

Weigl, E. 1941. On the psychology of so-called processes of abstraction. *Journal of Abnormal and Social Psychology,* 36**,** 3-33.

Weller, R. O., Massey, A., Newman, T. A., Hutchings, M., Kuo, Y. M. & Roher, A. E. 1998. Cerebral amyloid angiopathy: amyloid beta accumulates in putative interstitial fluid drainage pathways in Alzheimer's disease. *Am J Pathol,* 153**,** 725-33.

Weller, R. O., Boche, D. & Nicoll, J. A. 2009. Microvasculature changes and cerebral amyloid angiopathy in Alzheimer's disease and their potential impact on therapy. *Acta Neuropathol,* 118**,** 87-102.

Wentzel, C., Rockwood, K., MacKnight, C., Hachinski, V., Hogan, D. B., Feldman, H., Ostbye, T., Wolfson, C., Gauthier, S., Verreault, R. & McDowell, I. 2001. Progression of impairment in patients with vascular cognitive impairment without dementia. *Neurology,* 57**,** 714-6.

Werring, D. J. 2001. *Mechanisms of central nervous system damage and recovery in demyelinating and other neurological disorders : structural and functional magnetic resonance imaging studies.* PhD, University College London

Werring, D. J., Frazer, D. W., Coward, L. J., Losseff, N. A., Watt, H., Cipolotti, L., Brown, M. M. & Jager, H. R. 2004. Cognitive dysfunction in patients with cerebral microbleeds on T2*-weighted gradient-echo MRI. *Brain,* 127**,** 2265-75.

Werring, D. J., Coward, L. J., Losseff, N. A., Jager, H. R. & Brown, M. M. 2005. Cerebral microbleeds are common in ischemic stroke but rare in TIA. *Neurology,* 65**,** 1914-8.

Werring, D. J. 2007. Cerebral microbleeds: clinical and pathophysiological significance. *J Neuroimaging,* 17**,** 193-203.

Werring, D. J., Gregoire, S. M. & Cipolotti, L. 2010. Cerebral microbleeds and vascular cognitive impairment. *J Neurol Sci,* 299**,** 131-5.

Werring, D. J. (ed.) 2011. *Cerebral microbleeds: Pathophysiological to clinical practice,* New York: Cambridge University Press.

Wharton, S. B., Brayne, C., Savva, G. M., Matthews, F. E., Forster, G., Simpson, J., Lace, G., Ince, P. G., Medical Research Council Cognitive, F. & Aging, S. 2011. Epidemiological neuropathology: the MRC Cognitive Function and Aging Study experience. *J Alzheimers Dis,* 25**,** 359-72.

Whitehead, S. N., Cheng, G., Hachinski, V. C. & Cechetto, D. F. 2007. Progressive increase in infarct size, neuroinflammation, and cognitive deficits in the presence of high levels of amyloid. *Stroke,* 38**,** 3245-50.

Windham, B. G., Griswold, M. E., Shibata, D., Penman, A., Catellier, D. J. & Mosley, T. H., Jr. 2012. Covert neurological symptoms associated with silent infarcts from midlife to older age: the Atherosclerosis Risk in Communities study. *Stroke,* 43**,** 1218-23.

Wiszniewska, M., Devuyst, G., Bogousslavsky, J., Ghika, J. & van Melle, G. 2000. What is the significance of leukoaraiosis in patients with acute ischemic stroke? *Arch Neurol,* 57**,** 967-73.

Wolff, K. 1937. Untersuchungen und Bemerkungen zur Lehre von der hypertonischen apoplektischen Hirnblutung. *Virchows Archiv,* 299**,** 573-628.

Wong, K. S., Mok, V., Lam, W. W., Kay, R., Tang, A., Chan, Y. L. & Woo, J. 2000. Aspirin-associated intracerebral hemorrhage: clinical and radiologic features. *Neurology,* 54**,** 2298-301.

Wong, K. S., Chan, Y. L., Liu, J. Y., Gao, S. & Lam, W. W. 2003. Asymptomatic microbleeds as a risk factor for aspirin-associated intracerebral hemorrhages. *Neurology,* 60**,** 511-3.

Wu, Z., Mittal, S., Kish, K., Yu, Y., Hu, J. & Haacke, E. M. 2009. Identification of calcification with MRI using susceptibility-weighted imaging: a case study. *J Magn Reson Imaging,* 29**,** 177-82.

Yakushiji, Y., Nishiyama, M., Yakushiji, S., Hirotsu, T., Uchino, A., Nakajima, J., Eriguchi, M., Nanri, Y., Hara, M., Horikawa, E. & Kuroda, Y. 2008. Brain microbleeds and global cognitive function in adults without neurological disorder. *Stroke,* 39**,** 3323-8.

Yamada, M., Tsukagoshi, H., Otomo, E. & Hayakawa, M. 1987. Cerebral amyloid angiopathy in the aged. *J Neurol,* 234**,** 371-6.

Yamada, M., Itoh, Y., Otomo, E., Hayakawa, M. & Miyatake, T. 1993. Subarachnoid haemorrhage in the elderly: a necropsy study of the association with cerebral amyloid angiopathy. *J Neurol Neurosurg Psychiatry,* 56**,** 543-7.

Yamada, S., Saiki, M., Satow, T., Fukuda, A., Ito, M., Minami, S. & Miyamoto, S. 2012. Periventricular and deep white matter leukoaraiosis have a closer association with cerebral microbleeds than age. *Eur J Neurol,* 19**,** 98-104.

Yamamoto, H., Watanabe, T., Miyazaki, A., Katagiri, T., Idei, T., Iguchi, T., Mimura, M. & Kamijima, K. 2005. High prevalence of Chlamydia pneumoniae antibodies and increased high-sensitive C-reactive protein in patients with vascular dementia. *J Am Geriatr Soc,* 53**,** 583-9.

Yamamoto, Y., Akiguchi, I., Oiwa, K., Hayashi, M., Kasai, T. & Ozasa, K. 2002. Twenty-four-hour blood pressure and MRI as predictive factors for different outcomes in patients with lacunar infarct. *Stroke,* 33**,** 297-305.

Yamamoto, Y., Ihara, M., Tham, C., Low, R. W., Slade, J. Y., Moss, T., Oakley, A. E., Polvikoski, T. & Kalaria, R. N. 2009. Neuropathological correlates of temporal pole white matter hyperintensities in CADASIL. *Stroke,* 40**,** 2004-11.

Yamauchi, H., Fukuda, H. & Oyanagi, C. 2002. Significance of white matter high intensity lesions as a predictor of stroke from arteriolosclerosis. *J Neurol Neurosurg Psychiatry,* 72**,** 576-82.

Yarchoan, M., Xie, S. X., Kling, M. A., Toledo, J. B., Wolk, D. A., Lee, E. B., Van Deerlin, V., Lee, V. M., Trojanowski, J. Q. & Arnold, S. E. 2012. Cerebrovascular atherosclerosis correlates with Alzheimer pathology in neurodegenerative dementias. *Brain,* 135**,** 3749-56.

Yong, W. H., Robert, M. E., Secor, D. L., Kleikamp, T. J. & Vinters, H. V. 1992. Cerebral hemorrhage with biopsy-proved amyloid angiopathy. *Arch Neurol,* 49**,** 51-8.

Zekry, D., Duyckaerts, C., Moulias, R., Belmin, J., Geoffre, C., Herrmann, F. & Hauw, J. J. 2002. Degenerative and vascular lesions of the brain have synergistic effects in dementia of the elderly. *Acta Neuropathol,* 103**,** 481-7.